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**Clinical validation of near-patient assessment of
fluid status in dialysis patients**

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requirements of the University of Keele for the
degree of Doctor of Philosophy**

Abstract

Dialysis patients have an extraordinarily high level of cardiovascular morbidity and mortality. Volume expansion is common among this cohort and this is linked to markers of cardiac dysfunction, inflammation and low plasma albumin, all of which predict worse survival. Fluid status deteriorates over time due to loss of residual renal function (RRF) and progressive muscle wasting and currently there is a lack of effective or validated clinical tools to detect such changes that can inform clinical decision making given that gold standard methods are not practical for routine use.

This thesis first evaluates the role of albumin and fluid status of peritoneal dialysis (PD) patients and demonstrates that hypoalbuminaemia is an important determinant of tissue overhydration that is largely extravascular given the normal plasma volume. Attempts to 'dry out' such patients may precipitate hypovolaemic episodes and loss of RRF. The cardiac sub-analysis suggests that cardiac dysfunction, in particular heart failure with normal ejection fraction (HFNEF) is prevalent and associated with higher intravascular volume.

Next the potential of bioimpedance analysis (BIA) in the form of vector plots to detect the changing body composition in order to improve fluid management was investigated in a multi-centre randomised controlled trial. The clinical impact of this tool was small given the overall stability of fluid status in non-anuric PD patients but deterioration in anuric subject could be ameliorated; in the secondary cardiac analysis, patients in the intervention group seems to experience less damaged hearts. This study underlined the value of RRF in maintaining normal body composition and cardiac function. Lastly, the feasibility of

measuring lung water volume using breath analysis of deuterium concentration by flowing afterglow mass spectrometer (FA-MS) following mouth inhalation and intravenous injection of deuterium was explored. The study data vastly overestimated the predicted volume and alternative approaches are needed in the future. (300 words)

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Publications and presentations arising from this thesis

Peered review publications

- **Tan, B. K.**, D. Smith, P. Spanel and S. J. Davies (2010). "Dispersal kinetics of deuterated water in the lungs and airways following mouth inhalation: real-time breath analysis by flowing afterglow mass spectrometry (FA-MS)." J Breath Res **4**(1): 017109.
- **Tan, B. K.**, C. Chan and S. J. Davies (2010). "Achieving euvoemia in peritoneal dialysis patients: a surprisingly difficult proposition." Semin Dial **23**(5): 456-461.
- **Tan, B. K.**, S. J. Davies, P. Spanel and D. Smith (2012). "Injection of deuterated water into the pulmonary/alveolar circulation; measurement of HDO in exhaled breath and implications to breath analysis." J Breath Res **6**(3): 036005.
- **Tan, B. K.**, Z. Yu, W. Fang, A. Lin, Z. Ni, J. Qian, G. Woodrow, S. B. Jenkins, M. E. Wilkie and S. J. Davies (2015). "Longitudinal bioimpedance vector plots add little value to fluid management of peritoneal dialysis patients." Kidney Int. doi: 10.1038/ki.2015.294.
- John, B., **B. K. Tan**, S. Dainty, P. Spanel, D. Smith and S. J. Davies (2010). "Plasma volume, albumin, and fluid status in peritoneal dialysis patients." Clin J Am Soc Nephrol **5**(8): 1463-1470.

Oral presentations

- “Developing Bioimpedance (BIA) as a tool for fluid management in Peritoneal Dialysis Patients: A validation Study”- EuroPD Meeting Oct 2011 Birmingham.
- “Left Atrial Volume Index (LAVI) And Left Ventricular End Diastolic Volume Index (LVEDVI) May Not Reflect Overhydration In Peritoneal Dialysis Patients With Hypertensive Cardiac Injury” -Audit and Research Symposium 1st Annual Meeting University Hospital of North Staffordshire/Keele University. 26 November 2010
- “Body composition and body water studied using FA-MS and BIA”- SIFT conference ‘SIFT-MS; current and proposed applications’. ISTM Keele University 3 November 2010
- “Global longitudinal strain and chamber stiffness correlate with plasma volume in chronic kidney disease”- 13th Congress International Society for Peritoneal Dialysis July 2010 Mexico City and the Renal Association/British Renal Society conference May 2010 Manchester.
- “Validation of longitudinal vector analysis of body composition with Deuterium dilution in haemodialysis patients”- ASN Renal Week November 2009 San Diego and the International Society of Blood Purification Sept 2009 Stockholm.

- “Indices of cardiac dysfunction in peritoneal dialysis patients are associated with relative increases in intravascular volume rather than extracellular water relative to total body water”- EuroPD Oct 2009 Strasbourg and the World Congress of Nephrology Milan, Italy. May 2009.
- “Dispersal kinetics of deuterium in exhaled breath measured by flowing afterglow mass spectrometry, FA-MS, and total lung water (TLW) estimation”- International Society for Breath Odour Research/International Association for Breath Research conference Dortmund, Germany. April 2009
- “Lung water study-PhD project”- Post graduate symposium April 2008

Poster presentations

- “Developing Bioimpedance (BIA) as a tool for fluid management in Peritoneal Dialysis patients: A validation study” Renal Association and British Transplant Society conference June 2011 Birmingham
- “Left Atrial Volume Index (LAVI) and Left Ventricular End Diastolic Volume Index (LVEDVI) may not reflect overhydration in Peritoneal Dialysis patients with hypertensive cardiac injury” Audit and Research Symposium Day University Hospital of North Staffordshire and ASN Renal Week November 2010 Denver

- “Global longitudinal strain and chamber stiffness correlate with plasma volume in chronic kidney disease.” ASN Renal Week November 2009 San Diego
- “What additional impact does end stage renal failure (ESRF) have in patients with heart failure and normal ejection fraction (HFNEF)?” Renal Association and British Transplant Society conference April 2009 Liverpool and EuroPD Oct 2009 Strasbourg
- “Incremental impairment of left ventricular longitudinal strain and diastolic function associated with hypertension with or without left ventricular hypertrophy (LVH) in chronic kidney disease (CKD 5) and normal renal function” Renal Association and British Transplant Society conference April 2009 Liverpool and EuroPD Oct 2009 Strasbourg
- “Validation of longitudinal vector analysis of body composition with Deuterium dilution in haemodialysis patients” Renal Association and British Transplant Society conference April 2009 Liverpool
- “Indices of cardiac dysfunction in peritoneal dialysis patients are associated with relative increases in intravascular volume rather than extracellular water relative to total body water” ASN Renal Week Nov 2008 Philadelphia and Baxter Extramural Grant Meeting

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Dr Biju John initiated the study described in chapter 3 (Albumin, plasma volume and fluid status in PD patients). He applied and obtained the ethical approval, collaborated with radiologist and cardiologist, designed the patient information leaflet and consent form. My role in the study was to identify and consent potential study candidate, perform clinical measurements of body composition, administered ¹²⁵I-labeled albumin solution to measure plasma volume, sampling and processing blood, facilitate appointment for echocardiographic measurement, data collection, analysis and dissemination.

I was the main study coordinator for the multicentre, multinational, randomized controlled BIA guided fluid management in PD patient study in 3 UK centres and 1 Shanghai centre described in chapter 4. My roles and responsibilities include:

- (1) Application for the ethical approval from the National Research Ethics Service (NRES) using the online Integrated Research Application System (IRAS).
- (2) Purchase and distribution of BIA devices and accessories in all UK centres
- (3) Staff training of handling devices and system support.
- (4) Design of patient information leaflet and consent form.
- (5) Design of an electronic data capture system that showed real time bioimpedance vector plots and clinical decision making.
- (6) Identification and recruitment of study participants (in UHNS site).
- (7) Perform clinical measurement of body composition and blood sampling and processing.
- (8) Monthly update of recruitment numbers to the online research eportfolio system, review of adverse incidents and conduct regular quality assurance checks on all sites.
- (9) Facilitate echocardiographic measurement with transport arrangement and close collaboration with the cardiologist.
- (10) Preparation of deuterium mixture for total body water measurement.
- (11) Data collection, analysis and dissemination.

Dr Zanzhe Yu was the study coordinator for the Shanghai arm of the study with responsibilities include from item 6 to 11 as listed above. She has also contributed significantly in some of the complex statistical work.

I was the sole coordinator and operator for the study of lung water measurement using breath analysis of deuterium abundance by FA-MS indicated in chapter 6 and 7. I applied for and obtained the ethical approval from the Local Research Ethics Committee (LREC). I imported Deuterium Oxide of the highest grade purity from United States and negotiated with a local NHS pharmaceutical department (Stepping Hill Hospital, Stockport) to

perform the necessary quality assurance checks, dilution and sterilization so that it was fit for intravenous injection. As far as I am aware this represents the index case for the study of body composition in this country. I then designed the patient information leaflet and consent form to recruit 9 haemodialysis into the pilot study. I operated the FA-MS instrument to perform continuous breath analysis and injected the Deuterium mixture, closely supervised by Professor David Smith who acted as a control and for quality assurance purpose. I collected and processed the data and further statistical modeling was performed by Professor Patrik Spanel. I also performed all headspace analysis of Deuterium enriched blood samples using FA-MS for all total body water measurement described in this thesis.

List of abbreviations

¹²⁵ I-HAS	radio labelled human albumin solution
A-Wave	late mitral diastolic inflow velocity
AGE	advanced glycosylation end products
ANP	atrial natriuretic peptides
APD	automated peritoneal dialysis
BIA	bioimpedance analysis
BIVA	bioimpedance vector analysis
CKD	chronic kidney disease
CRP	C-reactive protein
D	Deuterium
DT	deceleration time of peak early Doppler mitral filling velocity
E-Wave	early mitral diastolic inflow velocity
E/A	ratio of early to late mitral inflow velocities
E/E'	ratio of early mitral diastolic inflow velocity to early diastolic mitral annular velocity
ECW	extracellular water
ESRD	end stage renal disease
EVLW	extravascular lung water
FA-MS	flowing afterglow mass spectrometry
FS	fractional shortening
GDP	glucose degradation products
GEDV	global End-Diastolic Volume
GFR	glomerular filtration rate
HD	haemodialysis
HFNEF	Heart failure with normal ejection fraction

IDH	intradialytic hypotension
ITBV	intrathoracic Blood Volume
ITTV	intrathoracic Thermal Volume
IVLW	intravascular lung water
IVRT	isovolumic relaxation time
IVSd	diastolic interventricular septal thickness
LAVI	left atrial volume index
LVEDDI	left ventricular end-diastolic diameter indexed to body surface area
LVEDVI	left ventricular end-diastolic diameter indexed to body surface area
LVH	left ventricular hypertrophy
LVMi	left ventricular mass index
PD	peritoneal dialysis
PMP	per million population
PSTR	peritoneal solute transport rate
PTV	pulmonary Thermal Volume
R	resistance
RRF	residual renal function
RRT	renal replacement therapy
TBW	total body water
TLW	total lung water
VPW	vascular pedicle width
Xc	reactance
Z	Impedance

Chapter 1 Introduction

1 Chapter 1 Introduction

1.1 End stage renal disease (ESRD): A significant health burden

1.1.1 The prevalence of ESRD

The kidneys perform many vital functions to maintain a constant optimal internal homeostasis in order to sustain life, one of which is the salt and water regulation. This is achieved by highly selective and tightly regulated filtration, reabsorption and elimination of water, electrolytes and solutes. Approximately 180 litres of plasma are filtered through the glomeruli everyday resulting in the production of around 1.5 litres of urine per day, indicating the highly efficient tubular reabsorption capacity. Other functions include maintenance of acid base balance, the production of erythropoietin essential for erythropoiesis, renin angiotensin system in blood pressure regulation and the metabolism of calcium, phosphate and vitamin D (Kerr, Bray et al. 2012).

Disruption of these important functions becomes evident as the glomerular filtration rate (GFR) falls below around $30\text{ml}/\text{min}/1.73\text{m}^2$. End stage renal disease (ESRD) or chronic kidney disease stage 5 (CKD 5) is defined by the estimated GFR of below $15\text{ml}/\text{min}/1.73\text{m}^2$ by the current UK guidelines (NICE 2014). It is characterised by the varying degree of abnormal fluid status, grossly deranged metabolic state with toxin accumulation, metabolic acidosis, renal osteodystrophy, inflammation, anaemia and malnutrition.

Such undesirable physiological disturbance can be partially ameliorated by the commencement of renal replacement therapy (RRT), which consists of haemodialysis (HD), peritoneal dialysis (PD) and renal transplantation. According to the 2014 UK Renal Registry 17th report, 7,006 patients commenced on RRT in 2013, reflecting the incidence rate of 109 per million population (pmp). There were 56,940 adults patients receiving RRT in the UK and the prevalence rate of RRT has increased from 523 pmp in 2000 to 888 pmp over the past 14 years. Transplantation is the most common treatment modality (52%) while HD was the more popular dialysis treatment (41.6%) compared to PD (6.4%) but the distribution varies greatly with age with younger patients tend to have a functioning kidney transplant while the older population treated with dialysis in particular HD (Figure 1.1 and Figure 1.2)

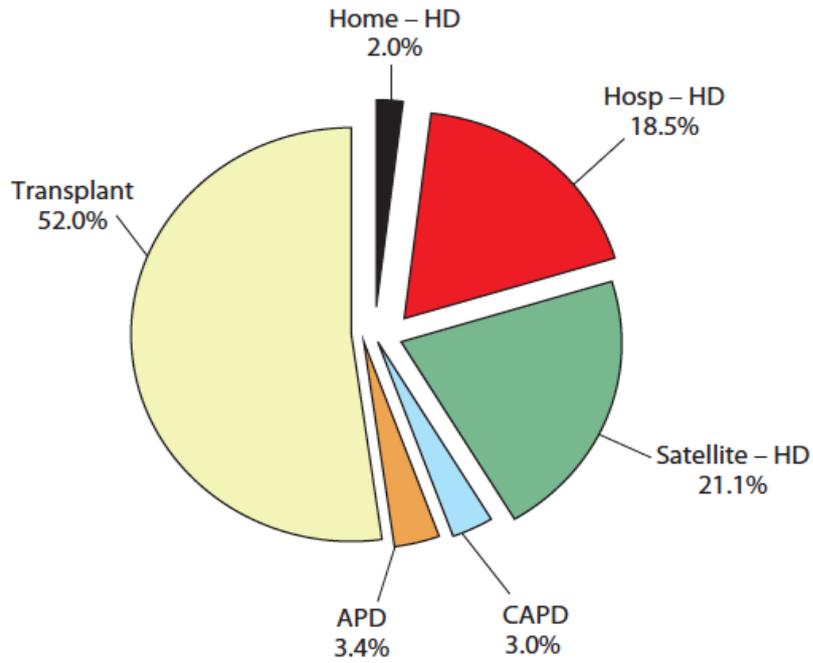


Figure 1.1 Treatment modality in prevalent RRT patients in 2013 (UK Renal Registry report 2014)

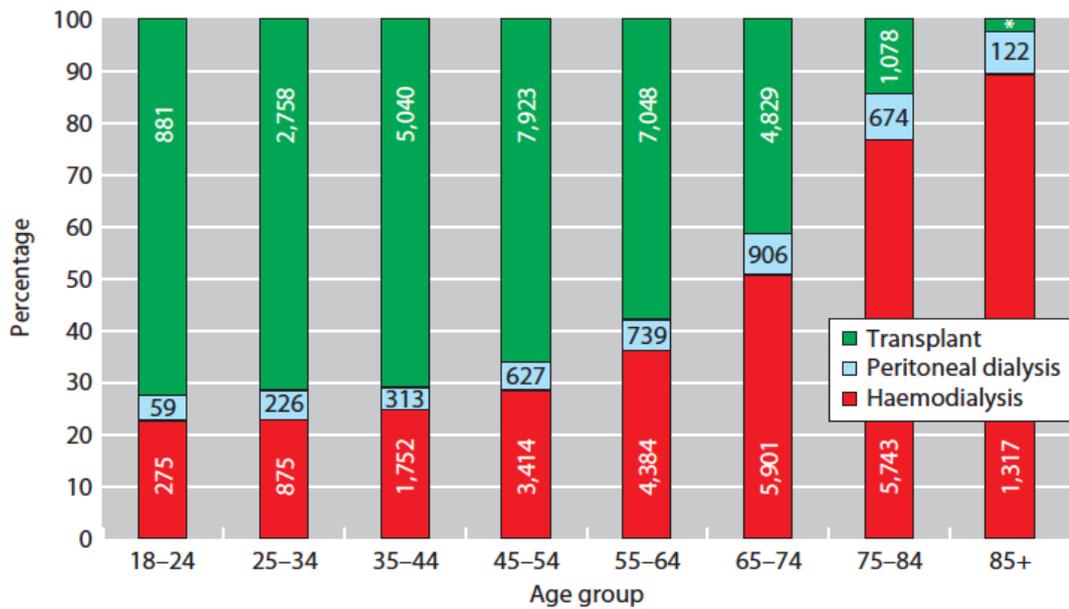


Figure 1.2 Treatment modality distribution by age in prevalent RRT population in 2013 (UK Renal Registry report 2014)

Although kidney transplantation confers better survival benefit and is considered preferred choice of RRT, the scarcity of donor kidneys and its prolonged waiting time coupled with

general frailty and multiple morbidities in the elderly CKD have compromised their eligibility. As the elderly is more susceptible to CKD, the incidence rate of CKD in this particular age group is forecasted to have the greatest increment given the overall increased survival of the general population (Figure 1.3).

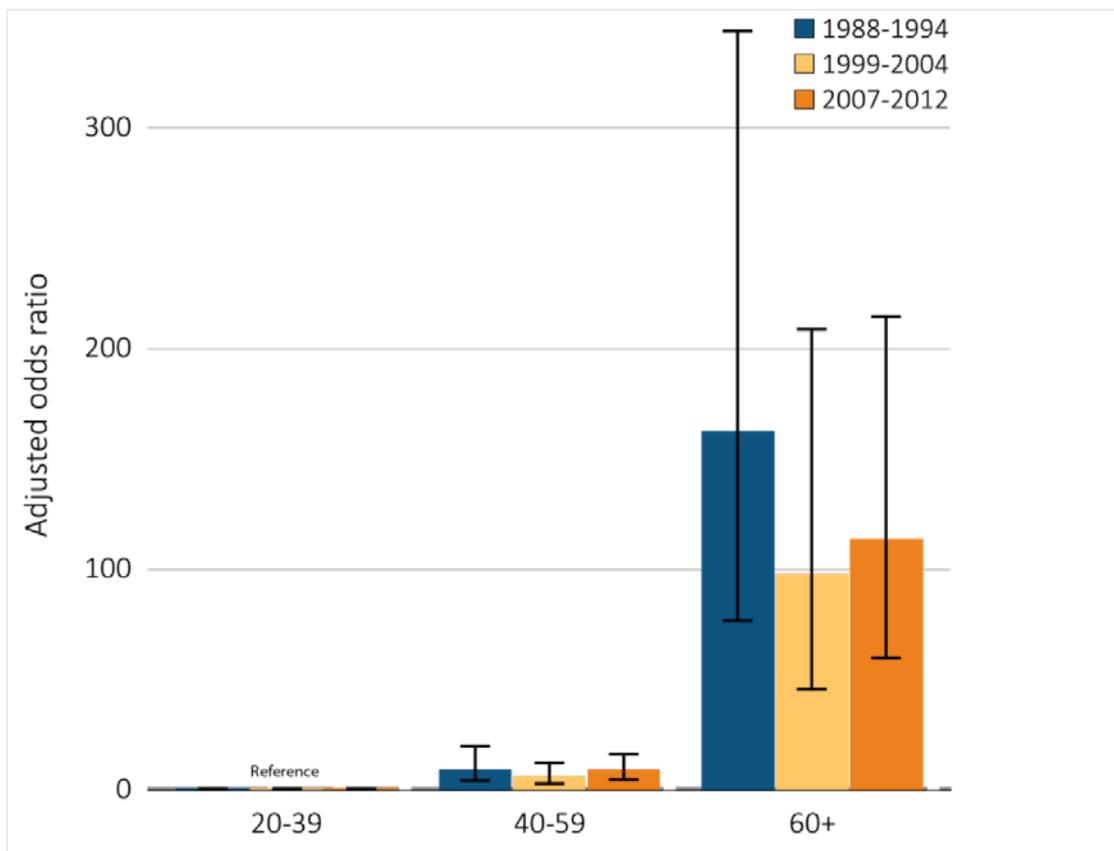


Figure 1.3 Adjusted odds ratios of eGFR<60 ml/min/1.73m² in National Health and Nutrition Examination Survey (NHANES) participants by age, 1998-2012

1.1.2 The health and financial cost of ESRD

Despite the declining trend in the overall mortality rate in RRT population over the past ten years in particular the 65 years and over, the survival is markedly reduced compared to the

general population without ESRD with the estimated 5 year survival rate of around 50% (Figure 1.4). The reason for such high mortality rate is multifactorial and likely to be attributed to patient factors (advancing age, frailty, increasing cardiovascular morbidity) and dialysis technique (cardiac stunning in HD). Cardiovascular disease was by far the commonest cause of death in the prevalent dialysis population that accounted for 27% of all reported deaths (Table 1.1) (Registry 2014). It was estimated that 7000 excess cerebral vascular disease events and 12,000 excess myocardial infarctions occurred in the CKD population, compared to the age and gender matched population without CKD at a cost of £174 to £178 millions to the NHS (Kerr, Bray et al. 2012). With the average annual cost of £23,287 per patient on RRT, this represented half of the £1.45 billions NHS spending on CKD care in 2009 to 2010, despite RRT only accounting for 2% of all CKD care (Baboolal, McEwan et al. 2008).

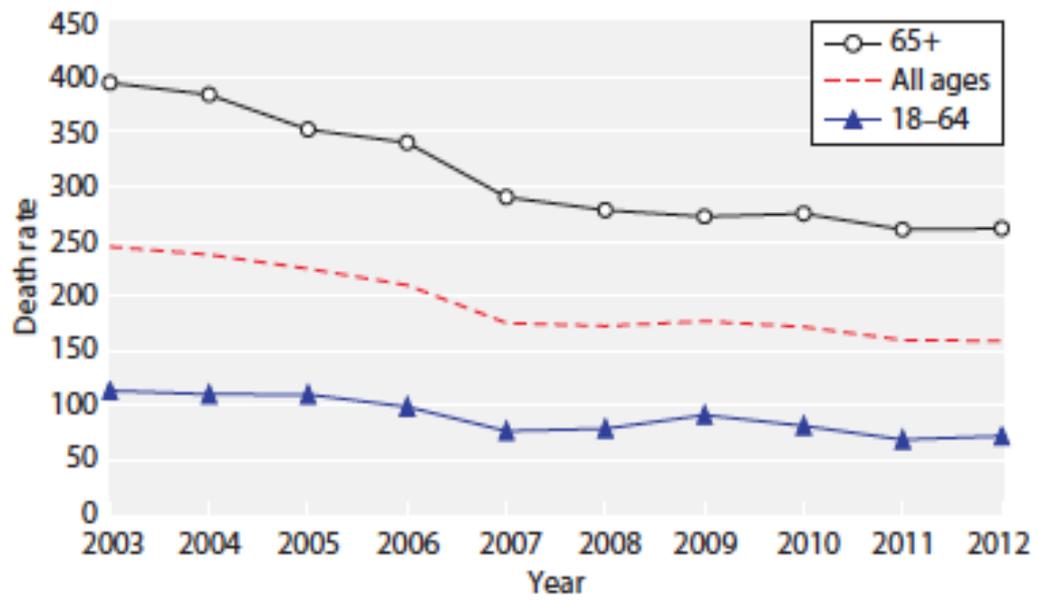


Figure 1.4 Death rate per 1,000 patient years in incident patients from 2003 to 2012 (UK renal registry report 2014)

Table 1.1 Cause of death in RRT patients by modality in 2012 cohort

Cause of death	All modalities		Dialysis		Transplant	
	N	%	N	%	N	%
Cardiac disease	734	23	647	24	87	17
Cerebrovascular disease	136	4	111	4	25	5
Infection	664	21	531	20	133	26
Malignancy	311	10	186	7	125	24
Treatment withdrawal	525	16	517	19	8	2
Other	660	21	543	20	117	23
Uncertain	186	6	161	6	25	5
Total	3,216		2,696		520	
No cause of death data	1,353	30	1,130	30	223	30

1.1.3 Dialysis principles and modalities

The Scottish chemist Thomas Graham first described the concept of dialysis in 1854 and this would form the basis of treatment for kidney failure. The main principle of dialysis is through the process of diffusion where different solutes are removed down a concentration

gradient across a semi-permeable membrane. They are two main types of modalities: haemodialysis (HD) and peritoneal dialysis (PD).

1.1.3.1 Haemodialysis

The first HD treatment of a uraemic patient was performed in 1924 by Haas in Germany using a filtering device made of collodion tubes. Hirudin was initially used to prevent clotting but it was unpredictable and subsequently replaced by heparin. There was progressive improvement in the design of dialyser and equipment throughout the 1940 and 1950 including the production of synthetic hollow fibre dialysers that greatly improved the efficiency of dialysis. In 1960 Quinton and Scribner invented an arterio-venous shunt made of silastic tubes with Teflon tips allowing continuous blood circulation during treatment. This was closely followed by the formation of native arterio-venous fistula first described by Cimino and Brescia, which remains to this day the safest and longest lasting vascular access.

Significant and continued improvements have been made in the subsequent decades in areas such as membrane biocompatibility, dialyser design, isothermal dialysis, safety measures and high flux membrane. Patients were previously vulnerable to unpleasant symptoms such as hypotension and headache following the exposure to the non-physiological acetate used as buffer. This issue is largely resolved with bicarbonate-based dialysis with dry bicarbonate concentrates. Further modification to the dialysis technique using convective process to increase solute clearance with high UF volume followed with fluid re-infusion called haemodiafiltration (HDF) and this is gaining popularity with the potential to offer greater haemodynamic stability during dialysis.

Despite the advancement of haemodialysis techniques over the recent decades, HD patients are still disadvantaged by very high rate of cardiovascular morbidity and mortality (Foley, Parfrey et al. 1998). Interventions to address conventional cardiac risk factors in this particular cohort have not produced the desirable results. This is perhaps not too surprising given atherosclerotic heart disease is not a predominant feature in HD patients. Alternative risk factors related to uraemia and dialysis technique have to be considered here. The pathogenesis of uraemic cardiomyopathy is complex involving structural remodelling at the macroscopic and cellular level culminating in measurable ventricular dysfunction (Gross and Ritz 2008). HD patients suffer from significant sudden cardiac death due to abnormal ventricle with arrhythmogenic potential. Apart from left ventricular systolic dysfunction, it is becoming more recognised that diastolic dysfunction occurs early in HD vintage (Silberberg, Barre et al. 1989, Levin, Singer et al. 1996). A combination of LVH and myocardial fibrosis results in reduced ventricular compliance and filling, giving rise to an increase in pulmonary pressure, predisposing patients to the development of pulmonary oedema.

There is a developing body of evidence that HD induces myocardial ischaemia and that this is determined by the amount and rate of UF and its associated haemodynamic instability during dialysis treatment (Zuber, Steinmann et al. 1989). The nature of conventional short intermittent thrice-weekly HD demands relatively high UF rate in a short space of time. This predisposes to intradialytic hypotension (IDH) when the UF rate exceeds the plasma refilling rate. This could be observed in 20 to 30% of HD patients and it predicts mortality (Bos, Bruin et al. 2000, Shoji, Tsubakihara et al. 2004). Myocardial stunning describes the phenomenon of acute reversible segmental myocardial

hypoperfusion causing regional systolic contractile dysfunction. Such repetitive ischaemic injury can lead to permanent systolic dysfunction (Selby and McIntyre 2007, McIntyre 2009). This can be studied using conventional intra-dialytic echocardiography to look for left ventricle regional wall motion abnormality (RWMA) which resolves upon termination of HD. Studies by Burton *et al* showed that up to 60% of HD patients developed myocardial stunning during dialysis treatment and it was independent predicted by IDH and UF volume in the multivariate analysis (Burton, Jefferies *et al.* 2009). In this 12 month follow up study, the 'non-stunners' were found to have only one significant cardiac event and 100% survival with no segmental shortening or reduction in LVEF. This was in stark contrast to 28% of the 'stunners' who died during the same period with the development of regional fixed systolic dysfunction and a significant 10% reduction in LVEF (Burton, Jefferies *et al.* 2009).

Apart from myocardial stunning, there are other cardiac parameters to support the concept of HD induced myocardial ischaemia. Studies suggest the rate of asymptomatic or silent ST depression on electrocardiogram (ECG) during HD is between 15 and 40% (Abe, Yoshizawa *et al.* 1996, Conlon, Krucoff *et al.* 1998). Singh *et al* used sestamibi single photon emission computed tomography (SPECT) as an alternative technique to evaluate coronary perfusion defects in ten HD patients without coronary artery stenosis. Seven patients were found to have perfusion defect but only three patients showed concurrent ST depression, indicating ST abnormality could have underestimated cardiac ischaemia (Singh, Langer *et al.* 1994).

In conclusion, HD treatment has undergone significant advancement and refinement over the years. This has enabled the achievement of desired solute clearance and ultrafiltration volume with relative ease. However, the detrimental effect of HD induced myocardial

ischaemia as a result of excess UF and IDH deserves careful consideration in order to limit long term irreversible cardiac damage and death.

1.1.3.2 Peritoneal Dialysis

In the early 1740s Christopher Warrick, an English surgeon presented his finding at the Royal Society meeting regarding the treatment of a 50 year old woman with ascites by instilling Bristol water and claret wine. This could be the first documented treatment involving the peritoneal membrane. This was followed by the first scientific description of the peritoneal cellular composition by Friedrich Daniel Von Recklinghausen. In 1877 the German G. Wegner discovered the basis of peritoneal ultrafiltration in the peritoneal cavity following the introduction of glucose containing solution. 45 years later, Putnam described the osmotic equilibrium between plasma and peritoneal fluid was driven by the concentration gradient across a semipermeable membrane through the process of diffusion.

George Ganter from Germany was critical of the extensive operation required by extracorporeal haemodialysis and he suggested that the peritoneal membrane could be exploited for the removal of uraemic toxins. Following some animal experiments he treated the first uraemic patient with PD in 1923 (Teschner, Heidland et al. 2004). Rhoads subsequently used lactate to correct acidosis and this became the standard buffer for PD.

By 1950, PD was used to treat over 100 patients (Odel, Ferris et al. 1950) but early technique was hindered by high rate of infective complication and the need to replace catheter frequently. Arthur Grollman and Morton Maxwell advocated a system where fluid

was instilled and let dwelt for a period of time before being drained out. The process can be repeated and this forms the intermittent basis of PD we observe today. With the development of the Tenckhoff catheter, a flexible silicone catheter with Dacron cuffs which could be inserted percutaneously, the introduction of the light titanium connector that reduced peritonitis rate and the availability of commercially produced closed system for easy drainage, the PD technique experienced a tremendous growth in its utilisation in the late 1970s and formed an integral part of home therapies.

It is estimated around 250,000 patients globally are currently on PD therapy, making it the commonest form of home dialysis (Jain, Blake et al. 2012). The technique's inherent simplicity enables patient self care for chronic disease management and promotes patient autonomy and control. Over the past two decades the survival of PD patients has steadily improved both in absolute term and in comparison with haemodialysis patients (Davies 2013). Most analyses have demonstrated equivalent medium term survival rate between three to five years between patients treated with PD and HD but early survival benefit is most apparent in patients with least comorbidity (Voges, Faict et al. 2004, Vonesh, Snyder et al. 2004, Kramer, Stel et al. 2009, Mehrotra, Chiu et al. 2009, Mehrotra, Chiu et al. 2011, van de Luijtgarden, Noordzij et al. 2011, Yeates, Zhu et al. 2012).

Despite the wide application of PD as a viable dialysis modality there are still a number of limitations both the clinicians and patients face. Glucose of varying concentrations has been used as an effective osmotic agent in the peritoneal dialysate to facilitate ultrafiltration since the beginning and it is still the case today. However the negative impacts of prolonged exposure of hypertonic glucose solution which also contains glucose

degradation products (GDP) on peritoneal membrane function has been well described, including the finding of advanced glycosylation end products (AGE) within the peritoneal membrane (Honda, Nitta et al. 1999) and the diabetiform and sclerotic changes in the peritoneal membrane (Hendriks, Ho-dac-Pannekeet et al. 1997, Krediet, Zweekers et al. 1999). Such morphological changes play a significant role in the alteration of membrane function characterised by rapid rate of peritoneal solute transport which results in the rapid absorption of glucose with the loss of osmotic gradient and fluid reabsorption. Early exposure to hypertonic glucose solution has been showed to be associated with increase in solute transport status while the use of low glucose strength (1.36%) is associated with stable membrane function over 5 years (Davies, Phillips et al. 2001).

There has been considerable work to develop a more biocompatible solution to address this issue and the introduction of Icodextrin (Extraneal®) in the 1980s represents a major improvement. Icodextrin consists of large molecular weight glucose polymer derived from starch that provides a more sustained osmotic gradient and ultrafiltration in the long dwell without the negative effect of glucose absorption. It is particular suited for patients with rapid peritoneal solute transport with reduced residual renal function and it has been showed to preserve peritoneal ultrafiltration and to improve fluid status with the reduction in ECW (Davies, Woodrow et al. 2003). However it is only licensed for once a day exchange and patients are still subjected to some degree of glucose exposure. Apart from the benefit of membrane preservation, it has been hypothesised that biocompatible solution may confer similar benefit in the maintenance of residual renal function (RRF). Anuric patients, both in HD and PD, tend to have more adverse cardiovascular, metabolic and volume status and more importantly the loss of RRF has been shown to be a power predictor of survival in two large randomised controlled trials (Bargman, Thorpe et al.

2001, Paniagua, Amato et al. 2002). Previous evidence did support the delayed onset of anuria with the use of neutral pH; lactate-buffered and low GDP solution compared with conventional solution but the decline in RRF did not reach statistical significance (Johnson, Brown et al. 2012). However, a more recent meta-analysis supports the idea of general use of biocompatible PD solution leading to greater urine output and higher RRF. Other clinical relevant benefits include better peritoneal ultrafiltration and avoid uncontrolled fluid overload (Cho, Johnson et al. 2014).

Peritoneal infection, together with ultrafiltration failure, remains an important cause of technique failure. Despite improvements in connection technology, peritonitis continues to be a major source of morbidity and mortality in PD patients with up to 18% of PD catheter removed and less than 4% resulted in death (Mujais 2006). While majority of peritonitis response well to antimicrobial treatment, patients are at risk of medium and long term sequelae including loss of RRF, peritoneal membrane injury with increase in solute transport and reduced ultrafiltration and the risk of developing encapsulating peritoneal sclerosis (Davies, Bryan et al. 1996). There is considerable variation in the incidence, practice and outcome in different PD centres and more studies are needed to understand this better. The likely strategy to reduce PD catheter associated infections probably lies in the adoption of best practice and evidence based approach such as regular patient education on exit site care, the use of prophylactic antibiotic during catheter insertion and the decontamination of nasal *Staphylococcus Aureus* with Mupirocin.

1.2 Cardiovascular disease in dialysis patients

Patients with ESRD treated with dialysis have disproportionately high morbidity and mortality rate and the leading cause of death is cardiovascular disease as previously alluded to (de Jager, Grootendorst et al. 2009, Collins, Foley et al. 2012, Registry 2014). The extent of the problem was best illustrated in a large epidemiological study comparing the cardiovascular mortality in dialysis patient and the general population across all age groups (Foley, Parfrey et al. 1998) (Figure 1.5) illustrated the overall major increase in death rate but the most dramatic relative increase was seen in the youngest population, hence patients who started dialysis in their 20s would have similar death rate as the over 80s without kidney failure.

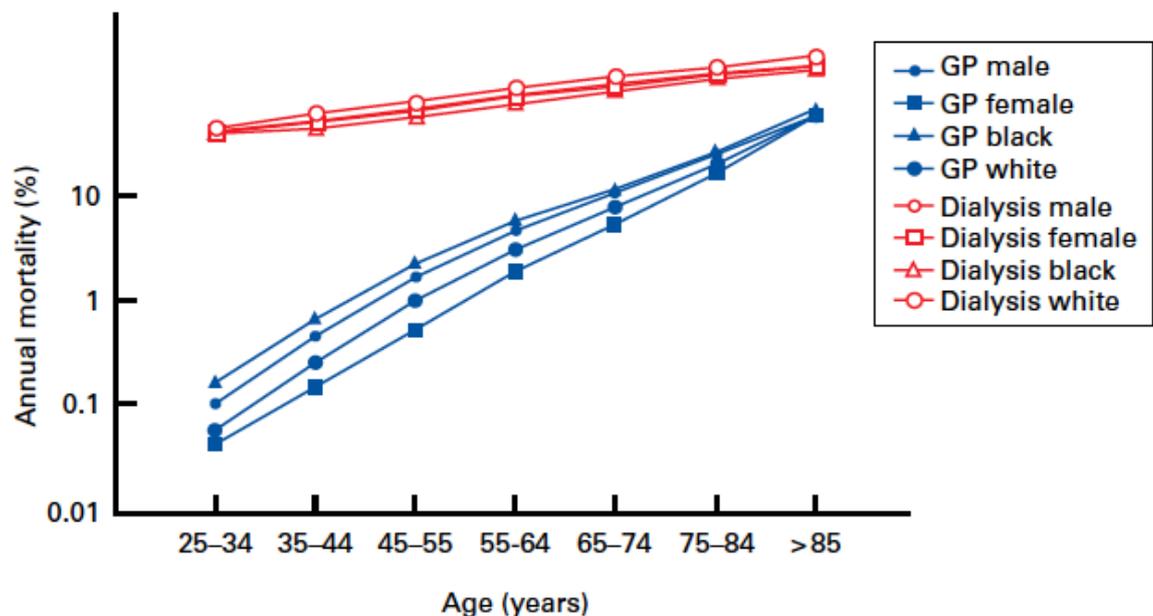


Figure 1.5 Cardiovascular death rate in the general population compared with patients on dialysis; USRDS data (Foley, Parfrey et al. 1998).

Dialysis patients share many 'traditional' cardiovascular risk factors with the general population such as diabetes mellitus, hypertension, dyslipidaemia, smoking etc. While

interventions to these risk factors have produced positive outcomes in the general population, this is not observed in the dialysis cohort. The recent SHARP (Study of Heart and Renal Protection) trial was a randomised double-blind multinational study to assess the effect of lowering LDL cholesterol with Simvastatin plus Ezetimibe in more than 9000 CKD patients of which over 3000 received dialysis therapy. Despite the reduction of LDL cholesterol and a significant 17% reduction in major atherosclerotic events, there was no impact on coronary or vascular mortality (Baigent, Landray et al. 2011).

1.2.1 Uraemic toxin

The cardiovascular death rate in dialysis population is therefore complex and multifactorial in nature and cannot be attributed entirely to the conventional cardiovascular risk factors. Hence the patients must be subjected to detrimental effects unique to uraemia and dialysis therapy in order to account for this increase in cardiovascular burden above and beyond than what is present in non-CKD patients. Uraemic compounds are toxic when they are biologically active but despite the identification of various toxins the mechanism as to how they contribute to the pathogenesis of cardiovascular disease is not well understood. More than 90 compounds have been compiled by the European uraemic toxin work group (EUTox) in 2003 based on their physicochemical characteristics that affect their removal by dialysis therapies (Vanholder, De Smet et al. 2003).

- Small molecules: They represent the majority of identified retained uraemic solutes. With molecular weight (MW) of ≤ 500 Da they are readily removed by dialysis and generally exhibit limited toxicity.
- Middle molecules: Characterised by MW of >500 Da. They have very low concentrations in uraemic patients but are toxic to multiple organs.

- Protein-bound molecules: Similar toxicity to middle molecules except smaller compounds with low MW.

Both middle and protein-bound molecules are not effectively removed by standard haemodialysis with small pores, low flux membranes compared with the water soluble small molecules (Locatelli, Mastrangelo et al. 1996). Peritoneal dialysis, on the other hand, offers better removal of the toxic compounds in part through the combination of semi-porous peritoneal membrane and the better preservation of residual renal function (Dhondt, Vanholder et al. 2000, Lameire, Vanholder et al. 2001). This may possibly explain the early survival benefit of PD over HD. There are evidence to support the pathogenic role of uraemic toxins in increasing the risk of cardiac morbidity and mortality in dialysis population, primarily in the acceleration of atherosclerosis and vascular calcification but so far effective therapeutic intervention remains elusive (Barreto, Barreto et al. 2009, Liabeuf, Barreto et al. 2010, Wang, Lu et al. 2010).

1.2.2 Inflammation and hypoalbuminaemia

Kidney failure is a pro-inflammatory state and chronic inflammation has been associated with increased cardiac death in dialysis population. An observational study in 91 HD patients showed that patients with the highest serum albumin had the best survival whereas the lowest survivors had the greatest CRP level (Yeun, Levine et al. 2000). Elevated plasma CRP level was found in 58% of PD patients in a single centre study and it was a powerful predictor of myocardial infarction during the 3 year follow up period (Herzig, Purdie et al. 2001). Compared with patients with normal level of CRP, raised level was associated with hypoalbuminaemia, reduced total weekly creatinine clearance and

increased left ventricular wall thickness. The predictive power of all cause mortality of raised CRP and hypoalbuminaemia was also established in CKD stage 3 and 4 prior to the initiation of dialysis treatment. The relationship between hypoalbuminaemia, inflammation and malnutrition is complex and may play a significant role in the acceleration of atherosclerosis, thus in part explaining the high cardiovascular death in this population.

1.2.3 Left ventricular hypertrophy

Left ventricular hypertrophy (LVH) appears early in CKD 3 and 4 and could be found in 60 to 80% of patients by the time of RRT commencement (Parfrey, Foley et al. 1996, Stack and Saran 2002). It is associated with patient's survival with multiple causative factors sharing a complex interaction. Pressure overload from hypertension and vascular calcification is a common finding in ESRD and predisposes the development of LVH. Renin-angiotensin-aldosterone system overactivity coupled with salt and fluid overload plays an important role in the pathogenesis of LVH (Yamakawa, Imamura et al. 2000). Abnormal metabolic profile with hyperparathyroidism (Fujii, Kim et al. 2007) and anaemia (Parfrey, Lauve et al. 2009) has been implicated as well. The myocardial changes in LVH are characterised by the increase in size of the myocardial cells, accumulation of interstitial collagen and fibrosis and the reduction of myocardial capillaries (Amann, Breitbach et al. 1998). Such pathological mismatch of hypertrophied myocyte and reduced capillary perfusion increased the risk of cell necrosis and this greatly enhanced the propagation of fatal cardiac arrhythmias (McLenachan, Henderson et al. 1987). This may partly explain up to 25% of all cardiac mortality is due to sudden cardiac death. LVH reduces left ventricle compliance that predisposes the development of pulmonary oedema when left ventricle filling pressure and left atrial pressure increase more readily in hypervolaemic

state. It also increases the risk of intradialytic hypotension in HD that has been identified as a strong predictor of cardiac death (Shoji, Tsubakihara et al. 2004).

1.2.4 Relationship between cardiovascular disease and abnormal fluid status in dialysis patients

Cardiovascular mortality in ESRD is strongly related to the presence of hypertension and LVH. There is cumulative evidence to implicate fluid overload as a significant driver of hypertension and LVH that accelerates cardiovascular disease that leads to reduced survival (London, Pannier et al. 2001, Zoccali, Benedetto et al. 2001). Overhydration as determined by multi-frequency BIA is an important independent predictor of mortality in maintenance HD patients (Wizemann, Wabel et al. 2009). The reduction of BP can be achieved with strict volume control without resorting to antihypertensive medication and this has led to the regression of LVH and improvement of arterial stiffness with possible survival benefit in HD patients (Onofriescu, Hogas et al. 2014).

Hypertension is extremely common in ESRD, affecting up to 80% of prevalent PD population with associated poor outcome (Cocchi, Degli Esposti et al. 1999, Koc, Toprak et al. 2002). However epidemiological study paints a complex picture regarding the relationship between hypertension and mortality with greater survival benefit in early stage of dialysis therapy and higher mortality in the longer term (Udayaraj, Steenkamp et al. 2009). Systolic BP of less than 110 mmHg was associated with reduced survival in prevalent PD cohort in an observational study and patients were more likely to have a history of heart failure, diabetes mellitus or receiving antihypertensive medication (Goldfarb-Rumyantzev, Baird et al. 2005). The pathophysiology of hypertension in ESRD

is complex involving the dysregulation of salt and water handling and overhydration is increasingly established as a major driver for hypertension in PD patients (Wang, Axelsson et al. 2005). There were initial concerns that fluid balance in PD patients may be less well preserved compared to other treatment modalities. ECW of 37% of PD patients was found to be above the 90th percentile compared to those with stable renal transplantation (Konings, Kooman et al. 2002) and markers of volume expansion such as high plasma atrial natriuretic peptides (ANP), increased left atrial volume and severe LVF were more common in PD than HD patients (Enia, Mallamaci et al. 2001). More circumstantial evidence to support overhydration in PD was the disproportionate use of antihypertensive medications (Velasquez, Lew et al. 1997, Cocchi, Degli Esposti et al. 1999, Enia, Mallamaci et al. 2001). However, careful examination of the data in the first direct comparison, using the bioimpedance analysis (BIA) derived ECW:TBW ratio, suggested that the fluid status of PD patients was similar to HD patients predialysis (Plum, Schoenicke et al. 2001). Interestingly the plasma ANP levels in PD patients were the same as HD patients postdialysis, despite their improved ECW/TBW ratio, which may suggest that compartmentalization of excess fluid may differ by treatment modality. With the introduction of icodextrin and the more widespread use of automated peritoneal dialysis (APD), especially in patients with rapid transport membrane characteristics, subsequent studies have consistently found that fluid status is not so different by treatment modality (Boudville, Cordy et al. 2007, Devolder, Verleysen et al. 2010, Paniagua, Ventura et al. 2010). It was estimated that as many as 25% (PD and HD) patients may have significant hypervolaemia (Devolder, Verleysen et al. 2010).

It is commonly acknowledged that PD is a more gentle dialysis therapy given its continuous ultrafiltration and solute clearance that can be performed at home. Therefore,

one would expect PD to be more cardio-protective but similar rate of cardiovascular death has been found between PD and HD. Glucose is the main osmotic agent used in conventional PD solution to drive ultrafiltration but it can be absorbed systemically to create multiple metabolic disturbances including hyperglycaemia, hyperinsulinaemia and fat gain which have negative impact on the cardiovascular risk factors (Holmes 2007). PD patients are not spared entirely from haemodynamic interruption as PD fluid exchange has been shown to cause increments in both systolic and diastolic blood pressure and total peripheral resistance proportional to the glucose strengths (Boon, Bos et al. 2001). The rapid disappearance of glucose in patients with rapid solute transport status and reduced ultrafiltration increases the risk of tissue overhydration, hypertension and multiple organ dysfunctions.

In view of such a unique profile the management of cardiovascular risk factors for PD patients differs from HD and requires careful consideration. First of all the loss of residual renal function is a strong predictor of all cause mortality including cardiovascular death (Wang, Wang et al. 2004). Increase in peritoneal small solute clearance was not associated with survival benefit suggesting peritoneal and renal clearance were not equivalent and qualitatively different (Paniagua, Amato et al. 2002). Therefore the preservation of RRF is considered an important strategy in PD patient care. The beneficial effect of angiotensin converting enzyme inhibitor and angiotensin receptor blockers on the rate of decline of renal function has been demonstrated in both diabetic and non diabetic patients likely attributed to the BP lowering effect (Casas, Chua et al. 2005). However, similar reno-protective effect was also seen in PD patients not explained by BP reduction (Li, Chow et al. 2003, Suzuki, Kanno et al. 2004). Furthermore, minimisation of glucose exposure is

desirable given the adverse effect of glucose on peritoneal membrane and metabolic profile.

1.2.5 Barriers to achieving euvolaemia in PD patients

Achieving a normal fluid status is therefore one of the prime objectives of RRT and PD is often regarded as a good therapy to achieve this. The combined benefits of a slow but sustained UF with relative preservation of RRF provide the optimal conditions to accomplish euvolaemic state. This is supported by evidence that shows that early survival in PD is excellent, probably superior to HD patients (Vonesh, Snyder et al. 2004), a benefit seen over a time frame likely to reflect salt and water management. Other studies have shown reductions in antihypertensive requirements and stabilization of LVH which coincides with these early survival benefits (Menon, Naimark et al. 2001).

However, longer term survival on PD is less favourable and there are several indicators suggesting that this is at least in part due to increasing difficulties with fluid management. First, inadequate ultrafiltration is an important cause of PD technique failure (Davies, Phillips et al. 1998). In observational studies, low peritoneal UF, especially in anuric cohorts is associated with reduced survival (Jansen, Hart et al. 2002, Brown, Davies et al. 2003, Paniagua, Amato et al. 2008). Interpretation of observational studies must be performed with care as low ultrafiltration volume could simply reflect poor dietary intake and hence a poor nutritional state.

Secondly, methods used to assess fluid status also predict survival. PD patients who have a reduced cell mass for a given overall fluid volume (implying excess ECW) as determined

by BIA technique have worse survival (Chen, Guo et al. 2007). This is also true for HD patients, emphasising the difficulties in maintaining satisfactory fluid status and salt and water balance in the context of changing body composition due to inflammation and muscle wasting. While this is not specific to PD it poses particular challenge in the regulation of fluid distribution because of the disproportionate hypoalbuminaemia seen with this modality.

Thirdly, there is a fine balance to strive for between euvolaemia and maintaining RRF. Well conducted observational cohort studies, e.g. NECOSAD (Jansen, Hart et al. 2002), have found that episodes of volume depletion predict more rapid loss of RRF independent of other known factors such as primary disease, comorbidity, diuretic use and RRF at the start of PD (Davies, Carlsson et al. 2009). In randomised trials comparing ultrafiltration achieved with icodextrin versus glucose, large reduction in ECW (regardless of the solution used) precipitantly caused a significant fall in RRF; in one study, four patients rendered anuric with icodextrin were clearly volume depleted on BIA analysis (Konings, Kooman et al. 2003, Konings, Kooman et al. 2005, Davies, Garcia Lopez et al. 2008). On the other hand, randomized trials of the newer biocompatible PD solutions have observed an increase in RRF in patients randomized to solutions that lead to less ultrafiltration because of an unexpected change in peritoneal membrane function (Davies 2009).

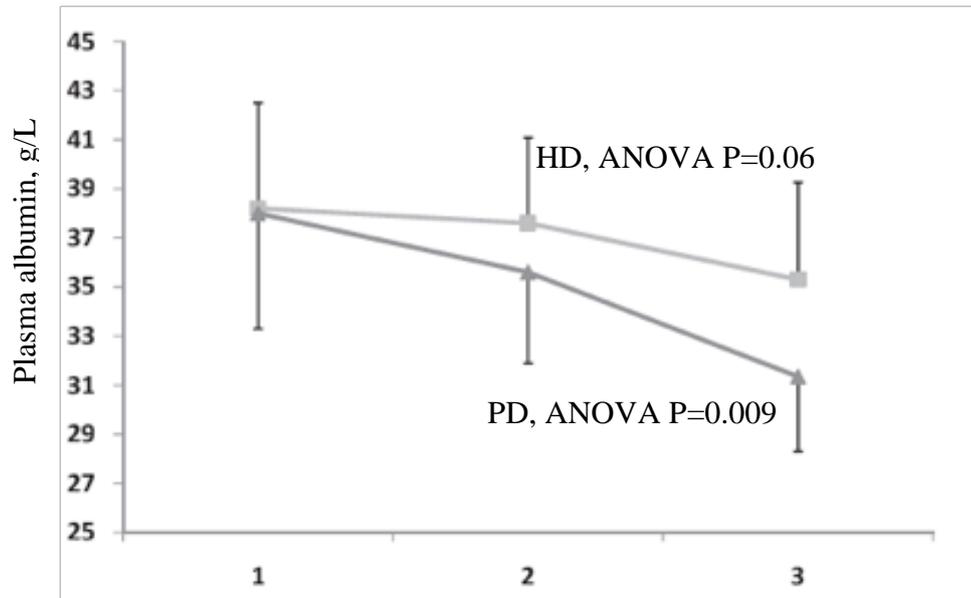
1.2.6 Albumin as a predictor of fluid status and survival

Albumin has an inverse relationship with ECW, a marker of tissue overhydration (Jones, Smye et al. 1998). In a 4-week intervention study to reduce dry weight by increasing peritoneal ultrafiltration, the reduction of ECW has resulted in a rise in serum albumin and

improved BP control in PD patients (Jones, Wells et al. 2002). On the contrary, a frequent versus normal haemodialysis frequency randomised trial which led to the reduction in ECW in the frequent group has failed to elevate albumin level (Kaysen, Greene et al. 2012). The negative charge acute phase protein also correlates inversely with inflammation and the interaction between hypoalbuminaemia, overhydration and inflammation has led to the speculation as a possible mechanism in the pathogenesis of malnutrition-inflammation-atherosclerosis (MIA) syndrome with negative cardiovascular prognosis (Avila-Diaz, Ventura et al. 2006).

There is a fundamental difference between HD and PD patients in the barrier to optimise fluid status and the fluid removal phenotypes. The problem with HD patients with significant cardiac dysfunction is that they repeatedly experience dialysis-induced hypotension thus frustrating attempts to achieve euvolaemia; HD patients with more than two comorbidities typically have three to five litre fluid excess post dialysis. Comorbidity also influences the pattern of longitudinal fluid gain, which is predominantly increased tissue hydration at the expense of lean tissue mass (Chan, McIntyre et al. 2009). Although worsening fluid status as determined by BIA is strongly correlated with a reduced plasma albumin in all dialysis patients (Jones, Akbani et al. 2002, Dumler 2003), this association is much stronger in PD patients (Figure 1.6). The single most important determinant of plasma albumin in PD is peritoneal protein loss. These losses are greatest in patients with larger, fast transport membrane especially when associated with significant comorbidity (Perl, Huckvale et al. 2009). In PD patients without comorbidity, increased protein synthesis largely compensates for this loss (Kaysen, Yeun et al. 1997). As comorbidity and inflammation increase, however, the ability to synthesize extra protein is impaired. This

has led to the hypothesis that hypoproteinaemia reduces plasma refilling and thus causes mal-distribution of fluid with excess extravascular ECW.



Tertiles of BIA derived ECW/TBW ratio (1=normal, 3=most abnormal)

Figure 1.6 Hypoalbuminaemia with worsening ECW/TBW ratio in different dialysis modalities. (▲=PD, ■=HD. Unpublished data)

1.3 Measurement of fluid status in dialysis patients using bioimpedance analysis (BIA)

Given the profound impact of hydration status has on patient’s survival through the mediation of hypertension, LVH, inflammation, nutrition and organ dysfunction, the determination of optimal fluid status of a patient and the subsequent detection of any deviation so that corrective measures can be put in place are considered essential clinical care. The approach is a clinical one after careful evaluation of physical examination, nutritional status and blood pressure. The so-called ‘dry weight’ is the ideal body weight where BP is within normal range without the aid of antihypertensive medication and patient is found to be oedema free. Peripheral oedema is a late physical sign that lacks

sensitivity and patient who is oedema free is certainly not synonymous with normohydration. This is especially true for younger patient population. Other physical signs that indicate hypervolaemia such as raised jugular venous pressure and respiratory crepitation can be difficult to discern and non-specific. The well tried and tested method of determining optimal dry weight in HD patients is to reduce the post dialysis weight until patients experience unpleasant side effects of hypovolaemia. This is less attractive in PD for a number of reasons; it is likely to compromise RRF as already discussed and necessitates use of hypertonic glucose, which may drive thirst and accelerate obesity which in turn further confuses the weight changes.

There is, therefore, a need for a more sophisticated measure of fluid status which would ideally discriminate between lean tissue mass, fat mass and ECW, preferably discriminating between intravascular and extravascular components of the latter. No such single tool exists. Those that have been used to evaluate fluid status in patients including BIA (Chen, Guo et al. 2007), echocardiography (Wang, Lam et al. 2006), brain/atrial natriuretic peptide (B/ANP) (Paniagua, Amato et al. 2008, Wang, Lam et al. 2009) may well have predictive value for both survival and episodes of fluid overload. However, none has so far been validated as a longitudinal management tool with clinically relevant endpoints. The general principle, techniques, strengths and weaknesses of BIA will be discussed here.

1.3.1 Bioimpedance Analysis-The general principle

Impedance (Z) refers to the degree of opposition of the alternating electric current (AC) of a conductor and is dependent on the frequency of the current. In the context of the human

body, it is determined by the vector relationship of resistance (R), which is inversely proportional to the amount of body water and reactance (X_c) or capacitance, which measures the ability of cell membranes to store electrical charges, hence proportional to the cell membrane mass (Jaffrin and Morel 2008). Theoretically, at low or zero frequency the current only passes through the extracellular fluid which yields the R of extracellular fluid (R_o). At infinite or very high frequency, AC passes through both the intracellular and extracellular water by penetration through the cell membrane which give rise to R_∞ . The relationship of the alternating electric current through different body compartments at different frequencies with their corresponding resistance and reactance values, can be demonstrated with Cole-Cole plot (Figure 1.7).

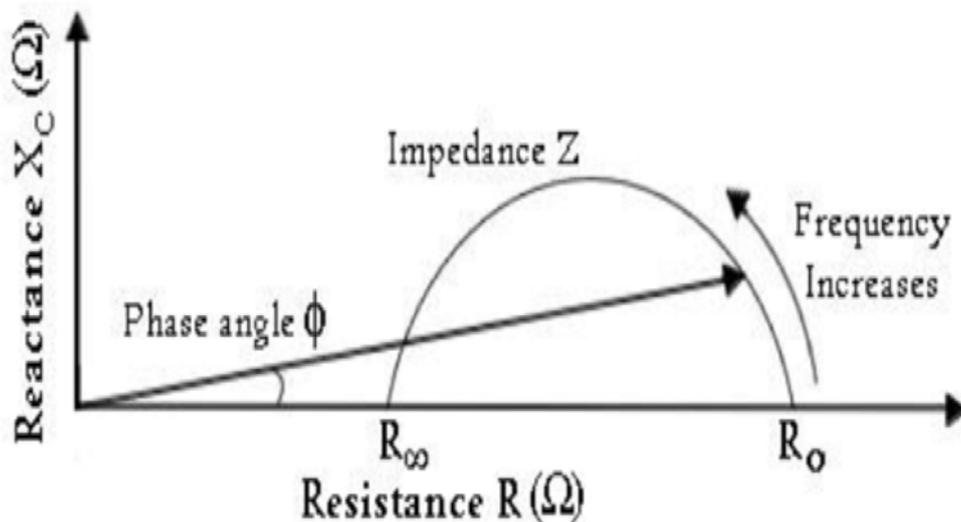


Figure 1.7 Cole-cole plot showing the relationship between resistance (R), reactance (X_c), impedance (Z) and the frequency of alternate current.

At low frequencies, the measurement of resistance is only confined to the extracellular resistivity as no current is passing through the intracellular compartment. As the applied frequency increases, the phase angle (Φ), which is the vector created by the impedance in

relation to resistance and can be calculated using the arctangent of the ratio of reactance to resistance (X_c/R), increases as more current is passing through the intracellular pathway. At very high frequencies, the current is entering both the intra and extracellular fluid compartment where capacitance becomes negligible and the reactance becomes zero and impedance is purely resistive. The relationship of resistance, reactance and impedance can be summarised by the equation.

$$Z^2 = R^2 + X_c^2$$

$$Z = \sqrt{R^2 + X_c^2}$$

$$\text{Phase angle } (\Phi) = \tan^{-1}(X_c/R)$$

The resistance (R) to electric current of an object is determined by the shape in terms of its length (L) and surface area (A). The bioimpedance model considers the human body as a cylindrical object and the relationship between resistance and the human body as the conductive material can be described with the following equations:

$$R = \rho L/A$$

Where ρ is resistivity given in $\Omega.m$ which measures the ability of a material to resist the passage of electric current for a defined unit of tissue. Reactance (X_c) is defined as resistance across the whole object and is inversely related to signal frequency (f) and capacitance (C) as described below:

$$X_c = 1/2 \pi f C$$

In biological tissue resistance is related to total body water and reactance to the capacitance of the body cell membrane.

1.3.2 Bioimpedance Vector Analysis (BIVA)

BIVA is a simple approach in monitoring fluid status by graphical representation of the two absolute measurements of resistance and reactance. Developed by Piccoli, the height adjusted resistance and reactance derived from the fixed frequency 50 kHz can be plotted on RXc graph as a vector which gives rise to phase angle and vector length (Piccoli, Rossi et al. 1994). The formulated 50%, 75% and 95% tolerance ellipses are based on the bivariate confidence limits for the healthy population and migration of the impedance vector in the left and right half of the 50% ellipse indicates the gain and loss of body cell mass whereas movement in the lower and upper half corresponds to increasing and decreasing fluid status (Figure 1.8).

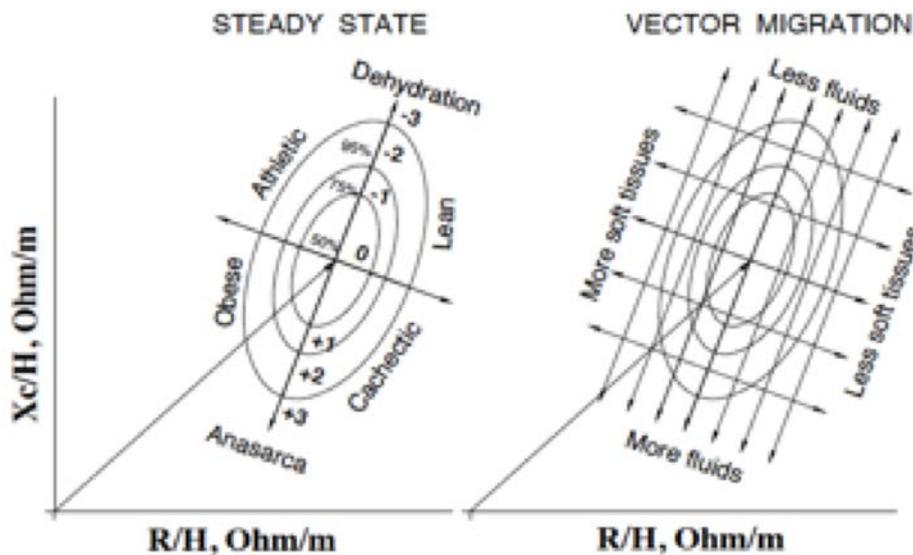


Figure 1.8 Piccoli's bioimpedance vector analysis in steady state and the significance of vector migration on body composition.

In the HD-BIA study, Piccoli *et al* compared the vector distribution of 1367 HD patients with 726 healthy populations (Piccoli 1998). The HD cohort was further subdivided to

stable asymptomatic group and recurrent HD hypotensive group. The wet-dry weight fluctuation of HD patients as a result of ultrafiltration was mirrored by the cyclical, backward-forward displacement of the impedance vector. The vectors of patients susceptible to HD induced hypotension were less steep and more often displaced out of the reference of the 75% tolerance ellipse than stable patients. In a similar study in PD patients the TBW estimation by anthropometric equations was misleading and failed to discriminate between oedematous and oedema free patients. However, vector distributions of both groups of patients were clearly distinctive with oedema free patients more similar to healthy population and oedematous patients close to vectors of nephrotic patients (Piccoli and Italian 2004). Such information can be used in the setting of optimal dry weight and adjustment of dialysis prescription to minimise cardiac decompensation in susceptible patients. In the study of over 3000 HD patients, Pillon *et al* has demonstrated shorter pre-dialysis bioimpedance vector which indicates tissue overhydration is associated with diminished survival in HD patients (Pillon, Piccoli et al. 2004). In PD, bioimpedance indexes correlates with nutritional markers and patients with phase angle of greater than or equal to 6 degree have better survival in observational study (Mushnick, Fein et al. 2003). Such predictive power of outcome has been replicated in other disease cohorts including HIV patients on highly active antiretroviral therapy (HAART) (Schwenk, Beisenherz et al. 2000) and advanced malignancies (Gupta, Lammersfeld et al. 2004, Gupta, Lis et al. 2004, Gupta, Lammersfeld et al. 2008). Large epidemiological study using BIVA involving over 15,000 children and 210,000 adults stratified by age and body mass index provides a useful healthy reference population for patient groups (Bosy-Westphal, Danielzik et al. 2005).

1.3.3 Single frequency and multi-frequency bioimpedance analysis

Single frequency BIA (SF-BIA) is the earliest proposed method to analysis body composition, usually at 50 kHz, mainly to predict the volume of TBW. Earlier study showed that H^2/R (known as resistance index where H is the height of subject) at frequency of 50 kHz has the best fit in regression analysis to the TBW measured by deuterium dilution method and this has been validated in subjects with normal hydration status but not in diseased states with deranged fluid status (Lukaski and Johnson 1985, Lukaski, Johnson et al. 1985, Lukaski, Bolonchuk et al. 1986).

Multi-frequency BIA (MF-BIA) involves the use of more than two frequencies in the analysis and determination of ECW and TBW using low and high frequency currents respectively based on the Cole model. Various limits of frequency have been proposed and some studies have incorporated isotope dilution techniques to validate ECW and TBW as determined by MF-BIA both in healthy subjects and dialysis patients (Chertow, Lowrie et al. 1995). One of the advantages of MF-BIA is the ability to discriminate ICW and ECW compartment with better accuracy in predicting ECW volume in healthy subjects (Simpson, Lobo et al. 2001) though some studies demonstrated the SF-BIA may offer better estimation of TBW in critically ill patients (Patel, Peterson et al. 1996).

Bioimpedance spectroscopy (BIS) is a more sophisticated model of MF-BIA using wide range of frequency and non linear mathematical models to estimate ECW, ICW and TBW, using normal population data as reference. Impedance data is used to calculate Absolute Delta Tissue Hydration ($A\Delta TH$) which represents the discrepancy between the actual amount of ECW in the tissue measured by BIA and the amount under normal physiological

state of the age and gender match healthy cohort. $A\Delta TH$ is further adjusted to ECW and the derived ratio is called Relative Delta Tissue Hydration ($R\Delta TH = A\Delta TH/ECW$). The 90th percentile of $R\Delta TH$ of the normal reference population is 7% and value exceeding 7% is considered fluid overload (FO) and if over 15%, severe fluid overload. One of the instruments that utilises the principle of BIS is the Body Composition Monitor (BCM, Fresenius Medical Care, Germany) which has been used in the monitor of fluid status in patients receiving dialysis treatments (Devolder, Verleysen et al. 2010, Machek, Jirka et al. 2010). The estimated fluid overload has been shown to predict mortality both in HD (Wizemann, Wabel et al. 2009, Chazot, Wabel et al. 2012) and PD (O'Lone, Visser et al. 2014) populations.

1.3.4 Strengths and weaknesses of BIA

There are many advantages of BIA, not least its non-invasiveness and easy portability but also highly reproducible measurements correlating fluid status and body cell mass. However, there are differing opinions regarding the optimal methodology between multiple frequencies BIA and single frequency, although the relationship between these two measures from which the hydration status is derived is highly correlated at every frequency. However determined, the R/Xc ratio is a strong predictor of survival such that patients with a relatively low reactance (tissue mass) in relation to resistance (body water) have a worse outcome.

It is also not clear how best to express the measured parameters to allow meaningful comparison in different treatment modalities with different body composition. The commonly used ratio of ECW/TBW ratio is often increased in PD patients compared with

healthy subjects because of the main abnormality in PD patients is an absolute reduction in tissue mass, reflecting their muscle wasting (Woodrow, Oldroyd et al. 2004). It is not clear whether the ECW/TBW ratio should be adjusted to account for the reduced lean tissue, although both PD and HD patients who were considered clinically euvolaemic had proportional reduction in ECW matching their reduced body water compared with healthy controls (van de Kerkhof, Hermans et al. 2004). There is also the issue regarding the extrapolation of absolute volume for TBW and ECW with impedance data using healthy population derived algorithms which assume normal tissue hydration index. This was clearly not the case as demonstrated in a longitudinal cohort of HD patients where the BIA estimated TBW volume was compared with absolute measurements using deuterium dilution; patients with more comorbidity were more overhydrated (Chan, McIntyre et al. 2009). This supports the observations that BIA tends to underestimate ECW the more overhydrated the patient is (Konings, Kooman et al. 2003). Finally, BIA is not able to discriminate ECW excess in intravascular from extravascular compartment. Clinical decision based solely on BIA derived hydration status could be erroneous without taking into account the patient's blood pressure, cardiac status and central volume, thus running the risk of compromising RRF.

Therefore, BIA is a promising tool in predicting outcomes and for monitoring longitudinal changes in fluid status and body composition as shown in interventional studies to modify hydration status in PD patients (Davies, Woodrow et al. 2003, Davies, Carlsson et al. 2009) but it has not been validated as a routine clinical tool and the optimal application of this technique requires further investigations.

1.4 Measurement of lung water volume

1.4.1 Why measure lung water volume?

The measurement of lung water volume has sparked much interest in the settings of critical care and renal failure. This is perhaps not surprising. Prognostically, extravascular lung water (EVLW) volume has been shown to correlate with survival in critically ill patients, with mortality rate of 65% in patients with EVLW > 15 ml/kg, compared with 33% in patients with EVLW < 10 ml/kg (Sakka, Klein et al. 2002)(Figure 1.9). In the same study EVLW has been established as an independent predictor of outcome (Figure 1.10). EVLW has been shown to predict clinical response to mechanical ventilation. Zeravik and co-workers have demonstrated patients with higher EVLW benefited from high-frequency ventilation whereas patients with subnormal or normal EVLW did well with pressure support ventilation (Zeravik and Pfeiffer 1989).

Study in HD patients revealed up to 67% of HD patients were found to have elevated lung water volume measured by thermodilution technique, indicating interstitial lung fluid excess (Kuhn, Kuhn et al. 2006). More worryingly, these asymptomatic patients did not exhibit any overt signs of pulmonary oedema and were considered clinically euvolaemia by their given target weight. The presence of subclinical pulmonary oedema HD patients was also confirmed by Wallin et al (Wallin, Jacobson et al. 1996) which was removed by dialysis, although no correlation was found between changes in lung water volume and body weight.

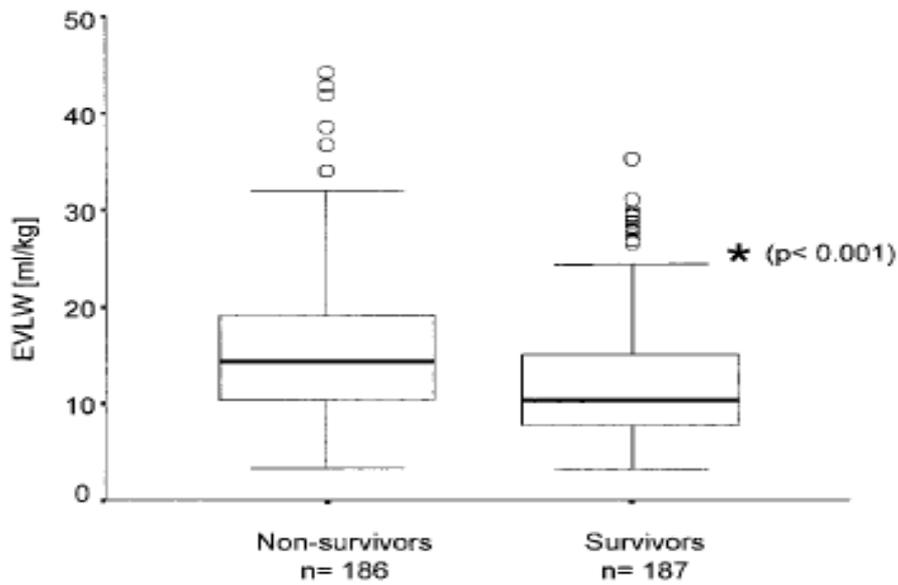


Figure 1.9 Using transpulmonary double indicators dilution technique to assess EVLW in the critically ill patients, non-survivors have higher EVLW volume.

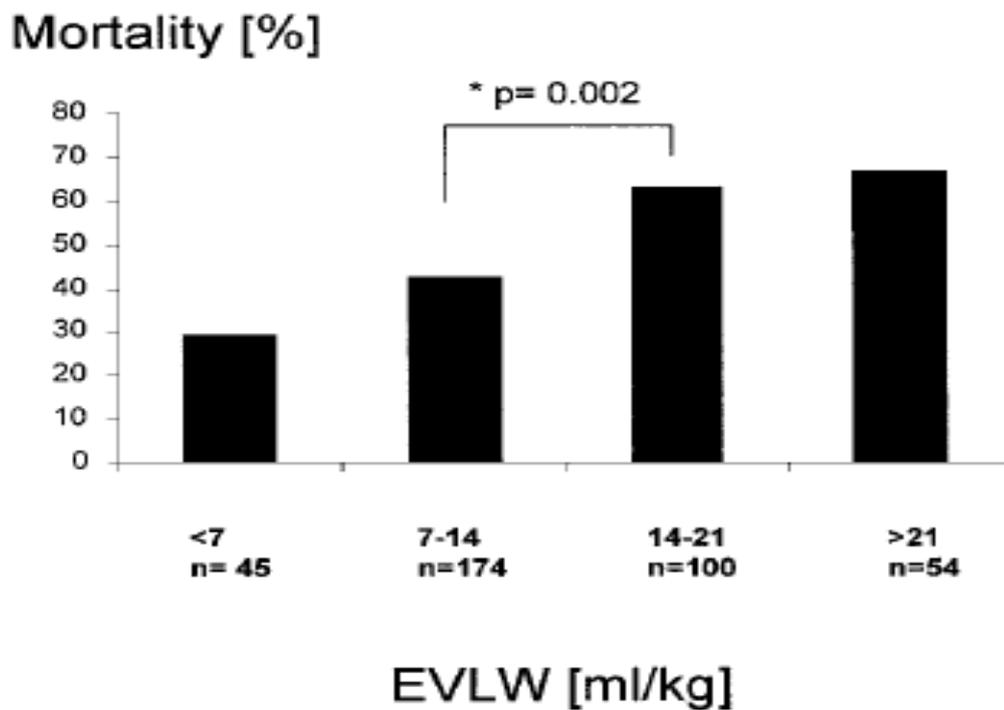


Figure 1.10 Mortality as a function of EVLW and patients were classified into four groups according to their highest EVLW volume.

The primary function of the lungs, which is to have a conducive environment to allow effective gaseous exchange in alveoli, the basic unit of the pulmonary system, is critically dependent on the protective mechanism to keep the gas filled air spaces relatively free from fluid. Pulmonary oedema is characterised by the abnormal accumulation of fluid in the extravascular spaces and may result from an increase in capillary permeability (acute lung injuries or acute respiratory distress syndrome) or from an increase in capillary pressure (increased hydrostatic pressure from cardiogenic pulmonary oedema). Such phenomena cause disturbance in the 'Starling equation' that favours the accumulation of EVLW which highlights the importance of the tight regulations between the hydrostatic and oncotic pressures in the intravascular and extravascular compartment.

$$\text{EVLW} = (L_p \times S) [(P_c - P_i) - \sigma(\pi_c - \pi_i)] - \text{lymph flow}$$

Where L_p =the hydraulic conductivity for water (cm/min/mmHg), S =surface area (cm²), P_c and P_i = the hydrostatic pressure in the capillary and interstitial spaces respectively, σ = the reflection coefficient for protein, π_c and π_i = the oncotic pressure within the capillary and interstitial spaces (mmHg).

The current gold standard to determine EVLW volume is post mortem examination using histological or gravimetric technique which clearly is not feasible for routine daily clinical use. Normal EVLW volume is approximately <500ml (Sivak, Starr et al. 1982, Sibbald, Warshawski et al. 1983, Gallagher, Moore et al. 1985) and physiological impairment occurs when the volume exceeds 75 to 100% (Bongard, Matthay et al. 1984). Pulmonary oedema can be detected clinically by chest auscultation and conventionally confirmed by

the findings of signs consistent with pulmonary congestion on chest radiograph but both methodologies are subjective and lack clinical sensitivity and specificity. There is a need to develop and validate techniques that are sensitive enough to detect the presence of subclinical pulmonary oedema and to monitor the effect of therapeutic intervention. The ideal test should be simple, non-invasive, accurate, sensitive, non-expensive and with highly reproducible result. Such single test does not exist but there has been a wide array of techniques aiming to fulfill the criteria. The current available clinical or research techniques can be broadly categorised into two main methodologies: imaging and indicator dilution.

1.4.2 Imaging methods

1.4.2.1 Chest radiograph

Chest radiography is a common and non-invasive tool used by clinicians to assess, among other cardiopulmonary pathologies, the presence of pulmonary oedema from the relatively well to the critically ill patients. It produces a visual estimation of excessive total lung water (intra and extravascular lung water) content or concentration. Certain radiographic features are considered to be characteristic of increased lung water volume such as pulmonary congestion, peribronchial cuffing, presence of septal lines (e.g. Kerley's lines) or air bronchograms, interstitial shadowing etc. As oedema increases, alveolar air space is replaced by fluid and the infiltrate becomes denser, particularly in the gravity dependent lung regions in mild to moderate pulmonary oedema and in severe cases all lung areas may be involved which may give a 'white-out' appearance. However, radiographic appearances of fluid, blood and inflammatory infiltrates are very similar and the density of infiltrates does not discriminate or infer the potential aetiologies.

The accuracy of chest radiograph to detect and monitor extravascular lung water has been evaluated in a series of studies using various double indicators dilution techniques. Pistolesi and Giuntini (Pistolesi and Giuntini 1978) studied the relationship between pulmonary oedema graded by a complex scoring system and EVLW measurement by ¹³¹I-labelled albumin and tritiated water as the non-diffusible and diffusible markers and found a significant but curvilinear correlation which makes clinical interpretation difficult. In a small study involving 14 critically ill patients, 5 patients were reported to have pulmonary oedema on radiological evidence though they have normal lung water measurement, indicating the problem of over-scoring in radiological examination (Sivak, Richmond et al. 1983)). Another study which utilized indocyanine green and ¹³¹I-labelled antipyrine as double indicators to evaluate EVLW found no correlation with cardiogenic and non-cardiogenic pulmonary oedema determined by radiographic interpretation (Liebman, Philips et al. 1978).

In the 1980's, Milne *et al* described the measurement of vascular pedicle width (VPW) which reflects the mediastinal silhouette of the great vessels on chest X ray (CXR) as a valid technique to measure intravascular volume (Milne, Pistolesi et al. 1984). It measures the distance between two points horizontally, first point formed by the crossing between the horizontal line and an imaginary vertical line dropped from the origin of left subclavian artery at the aortic arch (step 1 in Figure 1.11) and second point the crossing between superior vena cava and right main bronchus (step 2 in Figure 1.11) (Ely, Smith et al. 2001). Taken at the upright and postero-anterior position they reported the normal mean of VPW to be 48+/- 5 mm. However, there are variation in the measurements in patients with differing heights and body habitus. Other factors such as physical rotation, degree of

inspiration, upright or supine positions, previous thoracic surgery and trauma will have impact on the interpretation of VPW.

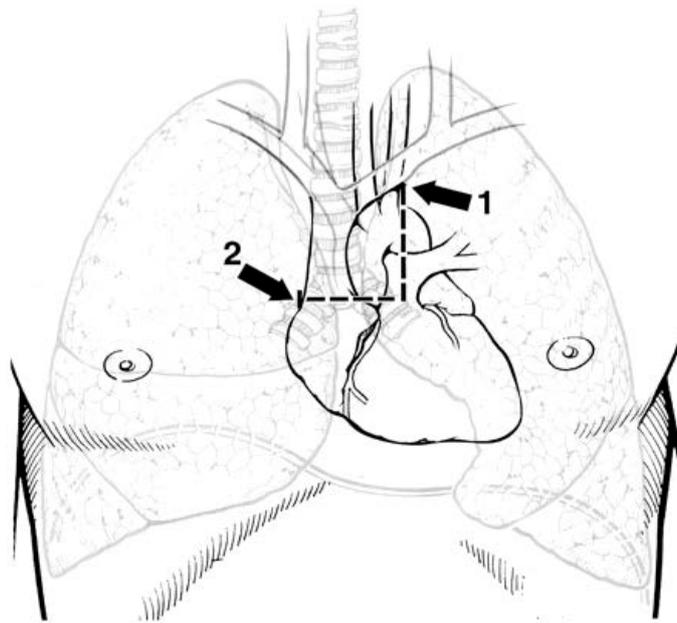


Figure 1.11 The vascular pedicle width (VPW) on chest X ray.

Much of the work of VPW measurement was conducted on mechanically ventilated patients in the critical setting. The two common challenging clinical scenarios faced by physicians are pulmonary oedema caused by acute respiratory distress syndrome (ARDS) from acute lung injuries and volume overload from cardiogenic or renal failure, both give similar CXR appearance. The differentiation between the two entities is crucial as they require different interventional therapies. In a series of clinical investigations Milne and coworkers found $VPW < 43 \text{ mm}$ was most predictive of patients with ARDS whereas an increased $VPW > 53 \text{ mm}$ was observed in hypervolaemic state from cardiac or renal failure (Figure 1.12) (Milne, Pistoletti et al. 1985).

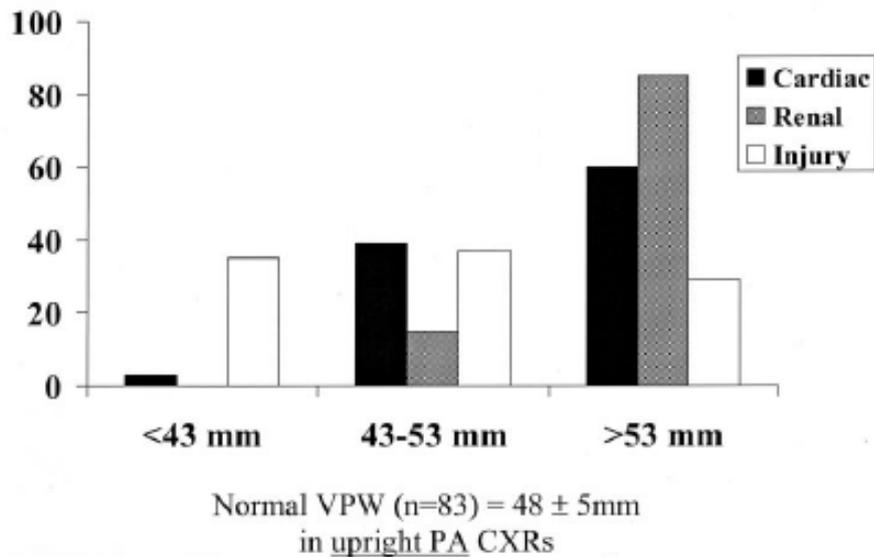


Figure 1.12 Vascular pedicle width in lung oedema.

Subsequent investigation by incorporating VPW and cardiothoracic ratio (CTR), another CXR measurement which assessed the ratio between the widest transverse diameter of the cardiac silhouette and the widest transverse diameter of the thorax above the diaphragm, improved the accuracy of CXR interpretation to differentiate between ARDS and volume overload by more than 30% (Thomason, Ely et al. 1998). These two radiological parameters correlate with pulmonary artery occlusion pressure (PAOP) which is generally considered to be the 'gold standard' of measuring intravascular volume. However this is done by Swan-Ganz catheter placement in the pulmonary artery and the safety and efficacy of such invasive procedure has been of considerable clinical concern. The clinical usefulness of VPW and CTR was examined using computer generated digital CXR in a prospective study of 100 critically ill patients who underwent pulmonary artery catheterisation. There was a modest increase in the radiologist's accuracy in discriminating volume status when clinical data was incorporated and VPW of > 70mm and CTR > 0.55

were the best cut off for differentiating a high intravascular volume status from a normal to low status (Ely, Smith et al. 2001). Although statistically significant, the association between VPW and pulmonary artery wedge pressure is weak and the lack of volumetric quantification means VPW cannot accurately predict intravascular volume status.

Overall, chest radiograph is a useful and non invasive tool which provides important information regarding lung density and possible aetiology of the disease process but its ability to predict lung water is seriously limited by difficult acquisition strategies in the critically ill patients and there is a lack of clinical validation in the day to day fluid management of dialysis population. It is also worth remembering that the interpretation of CXR findings in the clinical study is conducted by experienced radiologist under best circumstances, something that is not applicable in real life.

1.4.2.2 Lung ultrasound

The modality of ultrasound (US) imaging of the lungs has been shown to be of some value in discriminating pulmonary oedema from chronic obstructive pulmonary disease in patients presented with acute dyspnea (Lichtenstein and Meziere 1998). The resulting image that reflects excess extravascular lung water is the product of an artifact of hyperechoic reverberation between the pleural and the oedematous pleural septa. Such characteristic appearance resembles the flying comets, hence the term lung comets or more recently US B lines (Volpicelli, Elbarbary et al. 2012) that correspond with Kerley B lines observed in routine chest X-ray. This modality offers many attractive features which include non-invasiveness, easy application without the need of specialized operators and real time measurements.

When dialysis patients were subjected to lung ultrasound examination, most patients had evidence of moderate to severe pulmonary congestion but worryingly they were often asymptomatic (Enia, Torino et al. 2013, Zoccali, Torino et al. 2013). Even after fluid removal with ultrafiltration with haemodialysis, significant number of patients still has evidence of significant pulmonary congestion (Zoccali, Tripepi et al. 2013). US lung comets have been linked to important clinical outcomes in dialysis population. In a prospective observational study of 96 HD patients US lung comets scores were a significant predictor of mortality independent of bioimpedance derived parameter of fluid status (Siriopol, Hogas et al. 2013). In a cross sectional study of PD patients lung water measured by lung ultrasound has been shown to correlate independently with physical functioning by the Kidney Disease Quality of Life Short Form (Enia, Tripepi et al. 2012).

Pulmonary congestion is often considered to be a marker of overall volume status but such assumption lacks clinical validation, more so in the dialysis population who is often associated with abnormal fluid status. One study showed the lung comet score (B lines) is not significantly different in patients with overhydration, normal hydration status and underhydration as determined by BIA. Possible explanations for this finding are the high prevalent of heart failure among dialysis population that frequently associated with lung congestion and the phenomenon of increased lung permeability in uraemia that may not be volume related. Classical physical signs of volume overload such as lung crackles and tissue oedema have shown poor correlation with pulmonary congestion characterized by US B lines (Torino, Gargani et al. 2016).

1.4.2.3 Computed tomography (CT)

The density of lung tissue is the composite value of the relative proportion of air, blood, EVLW, and lung parenchymal and these elements can be quantitatively studied by the evaluation of the attenuation of electron beam with CT. In an experimental animal study, CT densitometry technique was able to detect a 69% increase in lung density from baseline when lung water weight was increased 250% gravimetrically (Hedlund, Vock et al. 1984). In a small study involved 10 HD patients, changes in lung fluid with ultrafiltration was reflected by changes in lung density and this corresponded with better alveolar ventilation and improved pulmonary function tests (Metry, Wegenius et al. 1997). Further study in HD also showed lung density measured by CT densitometry correlated with the measurement of the diameter of inferior vena cava (IVC) which is closely related to central blood volume and total body water leading to the hypothesis that normalisation of lung water is a good indication of dry weight in HD patients. However, CT instruments are not easily portable and involve potential harmful ionizing radiation, limiting its use within experimental setting.

1.4.2.4 Nuclear magnetic resonance (NMR) imaging

NMR is another modality that can be used to measure total lung water due to the abundance of proton in the water by the alignment of hydrogen nuclei of water molecule which can be manipulated with external magnetic field (Mayo 1994). The spin-echo sequence is the only one technique used in the measurement of lung water. A number of studies have reported good correlation between measurement of lung water by NMR and the gold standard gravimetric method (Cutillo, Morris et al. 1986, Phillips, Allen et al. 1989, Caruthers, Paschal et al. 1998). However, NMR may underestimate lung water by as

much as 40% in mild pulmonary oedema due to relatively little signal produced by conventional spin-echo sequences on 1.5 Tesla imagers. The issue can be resolved with the development of a multi-echo sequence with a much less powerful magnetic field (0.15 Tesla). This showed good correlation with the gravimetric method and this was subsequently validated in human subjects (Mayo, MacKay et al. 1995).

One of the advantages is the ability to measure intravascular lung water (IVLW) volume by evaluating the signal strength before and after the administration of the contrast medium such as polylysine-(Gd-DTPA). Extravascular lung water can be obtained by subtracting IVLW from TLW. Another advantage is the possibility to study the potential aetiology of pulmonary oedema. Due to the different signal intensity emitted by different tissues and subsequently acquired at longitudinal relaxation time (T1) and transverse relaxation time (T2), it is possible to study the difference in the composition EVLW in hydrostatic pulmonary oedema in heart failure (low protein content) and permeability pulmonary oedema in ARDS (high protein content).

NMR faces technical challenges as it is susceptible to artifact from chest wall motion during respiration. Certainly, the avoidance of ionizing radiation makes it an attractive option, but it is expensive, difficult to apply in critically ill or obese patients and contraindicated in patients with implanted magnetic materials.

1.4.2.5 Positron emission tomography (PET)

PET is widely considered to be the gold standard for the measurement of EVLW. A sample of sterile water labeled with a positron-emitting isotope such as oxygen -15 ($H_2^{15}O$) with a

half-life of approximately two minutes is administered intravenously. The radioactive labeled tracer is given time (three to four minutes) to equilibrate within the intravascular and total water compartment of the lung. The emissions are detected by an external device such as gamma camera or a PET scanner and the isotopic activity concentration can be determined in the lung tissue. The PET image can be used as a quantitative map of lung water distribution. A separate approach is needed for the estimation of EVLW volume. First, a standard acquisition of the PET image which contains total lung water distribution is performed as described above. Second, trace amounts of $C^{15}O$ gas is inhaled and it binds readily with blood haemoglobin. Following the equilibration with the total blood volume after a given time another PET image is performed. Assuming 84% of blood is water with normal haematocrits, the intravascular water content of the lung can be deducted from the total lung water concentration, giving rise to EVLW concentration (Schuster, Marklin et al. 1986).

The measurements of EVLW by PET correlated very well with the one obtained by gravimetrics. The measurements are highly reproducible with coefficient of variation of less than 5% and extremely sensitive to detect even a small increment in extravascular water (Schuster, Marklin et al. 1986). However, despite the superior qualities over the other modalities, PET scan is expensive, not widely available and involves ionising radiation. These severely limit its general application in general clinical setting.

1.4.2.6 Electrical impedance tomography (EIT)

The basic principle of EIT is based on the presence of air, fluid and lung tissues offering various resistances which can be measured by bioimpedance analysis when alternating electric current of fixed frequency is applied in the thoracic region. Such measurements

have been shown to correlate with gravimetric EVLW adjusted by weight (Spinale, Reines et al. 1989, Zellner, Spinale et al. 1990, Nierman, Eisen et al. 1996). In animal studies EVLW estimated by EIT and double indicators dilution technique showed good correlation with high reproducibility (Spinale, Reines et al. 1989). Lung impedance changed in response to diuretic treatment in patients with congestive heart failure where a significant increase of 8% of the mean lung resistivity was observed indicating dehydration of the lungs, offering the potential to guide medical intervention of this common condition (Noble, Harris et al. 2000). In haemodialysis population, total body impedance (TBI) measurement has been used to reflect total body water (TBW) changes. However, the extremities are responsible for 80% of the signal measured, making TBI a poor indicator of thoracic fluid content. During haemodialysis session both TBI and thoracic impedance (TI) increased with ultrafiltration, indicating a decrease of TBW and thoracic fluid though patients were considered to be euvolaemic and free from overt pulmonary oedema (Vonk Noordegraaf, van der Meer et al. 1995). This is partly due to UF induced hypovolaemia precipitating a reduction in thoracic blood volume and pulmonary perfusion. The value of EIT in patients undergoing peritoneal dialysis has not been investigated before.

There are several factors that need to be taken in account in the interpretation of thoracic impedance. The resistance increases with albumin concentration, a phenomenon seen *in vitro* studies and subsequent human study has showed impedance measurements differ in cardiogenic (low protein state) versus non-cardiogenic (high protein state) pulmonary oedema which gave rise to different lung water measurements (Raaijmakers, Faes et al. 1998). Both the variation in tidal volume and density of lung parenchyma may affect the analysis of electrical impedance in the thorax. Ventilatory-induced impedance changes with tidal volume and patients with emphysema show a significantly lower impedance

change between inspiration and expiration in comparison with healthy controls (Kunst, Vonk Noordegraaf et al. 1998). Despite the limitations, electrical impedance of the thorax is a safe, easy to apply, non-invasive and inexpensive technique which makes it clinically attractive but it has not been validated as a useful clinical tool to monitor lung water volume.

1.4.3 Indicator dilution methods

The indicator dilution methods are first described in the 1950s based on mathematical concepts and models (Newman, Merrell et al. 1951, Meier and Zierler 1954). When a known volume of indicator is injected into a central vein such as the internal jugular vein via a central venous catheter and blood is simultaneously sampled at a remote arterial site, commonly the femoral artery, the concentration of the indicator can be determined. The product of the flow through the system (cardiac output) and the mean transit time (obtained from the dilution curve) allows the calculation of the total volume of distribution of the indicator. The use of a freely diffusible water indicator (D_2O) or a thermal indicator (cold saline) would give rise to volume of distribution that includes both the intravascular and extravascular lung water. With the incorporation of a non-diffusible indicator (indocyanine green dye) which is restricted to the intravascular compartment and can be detected simultaneously in the arterial system, a different dilution curve emerges and EVLW volume can be estimated by comparing the volume of distribution of the two indicators. This is known as the double-indicator method.

The use of a single thermal indicator to estimate EVLW volume in humans was performed in the late 1980s requiring the insertion of pulmonary artery catheter and femoral arterial

catheter with a thermistor (Baudendistel, Kaminski et al. 1986). It also provides other important clinical parameters such as cardiac output, central venous pressure and pulmonary wedge pressure but the wider application is limited by its invasiveness and inherent high risk of complications. Moreover, it significantly overestimated EVLW volume by as much as 20% in a comparable study with double indicator method (Schuster and Calandrino 1991). By using a different approach, Sakka and co-workers evaluated the use of a single cold indicator to assess EVLW volume in a cohort of critically ill patients (Sakka, Ruhl et al. 2000) based on the physiological relationship between Global End-Diastolic Volume (GEDV) and Intrathoracic Blood Volume (ITBV). The technique is called pulse-induced continuous cardiac output (PiCCO) and the method of measurement can be described as follows: After the injection of a bolus of cold saline, the total volume of distribution of the cold indicator is obtained as the product of cardiac output and mean transit time, which is termed as the Intrathoracic Thermal Volume (ITTV). The pulmonary Thermal Volume (PTV), which is the largest chamber for the equilibration of cold indicator with lung water can be calculated by multiplying the cardiac output with the exponential down slope time of the thermodilution curve. The GEDV is the difference between ITTV and PTV. Using the study series, the Intrathoracic blood volume (ITBV) can be predicted from GEDV measurements with high accuracy using the equation $ITBV = (1.25 \times GEDV) - 28$. The discrepancy between ITTV and ITBV would give rise to the EVLW volume (Figure 1.13) (Michard 2007). Although different equations have been reported in other human studies, the derived EVLW volumes by transpulmonary thermodilution technique were quite similar and shown to correlate closely with the values obtained by the double-indicator dilution technique (Figure 1.14) (Reuter, Felbinger et al. 2002, Michard, Schachtrupp et al. 2005).

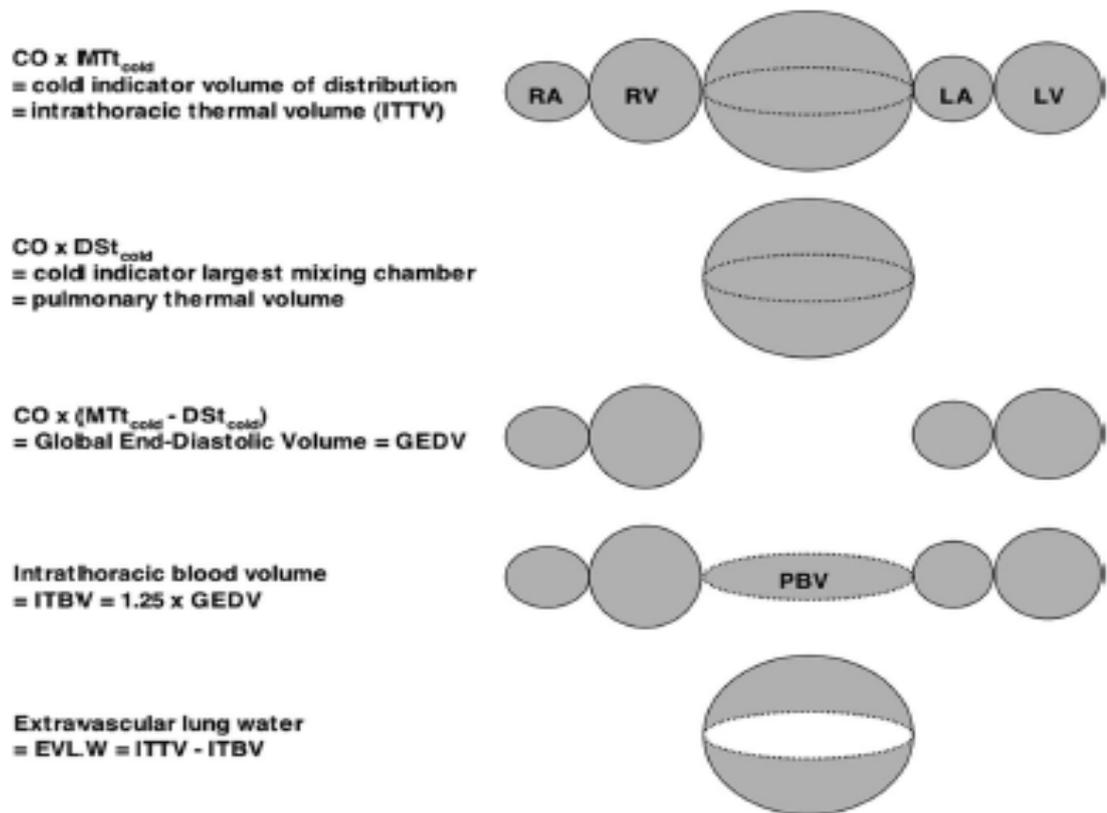


Figure 1.13 Principles of extravascular lung water (EVLW) estimation by the single-indicator dilution method. The intrathoracic blood volume (ITBV) is derived from the measurement of the global end-diastolic volume (GEDV), see the above equation. EVLW is the difference between the total volume of distribution of the thermal indicator and ITBV. CO, cardiac output; MTt, mean transit time; Dst, down slope time; RA, right atrium; RV, right ventricle; PBV, pulmonary blood volume; LA, left atrium; LV, left ventricle.

PiCCO is a device capable of measuring several important clinical parameters including continuous cardiac output, cardiac preload and systemic vascular resistance. It can be performed via a central venous line and an arterial catheter with a thermistor in one of the larger systemic arteries such as the femoral artery, something not uncommonly found in a typical patient in intensive care unit. It does not require any other additional access and real time measurement of cardiac output can be obtained. However, it is still an expensive device and repeated bolus injection of cold saline for calibration is important in ensuring accuracy of measurement.

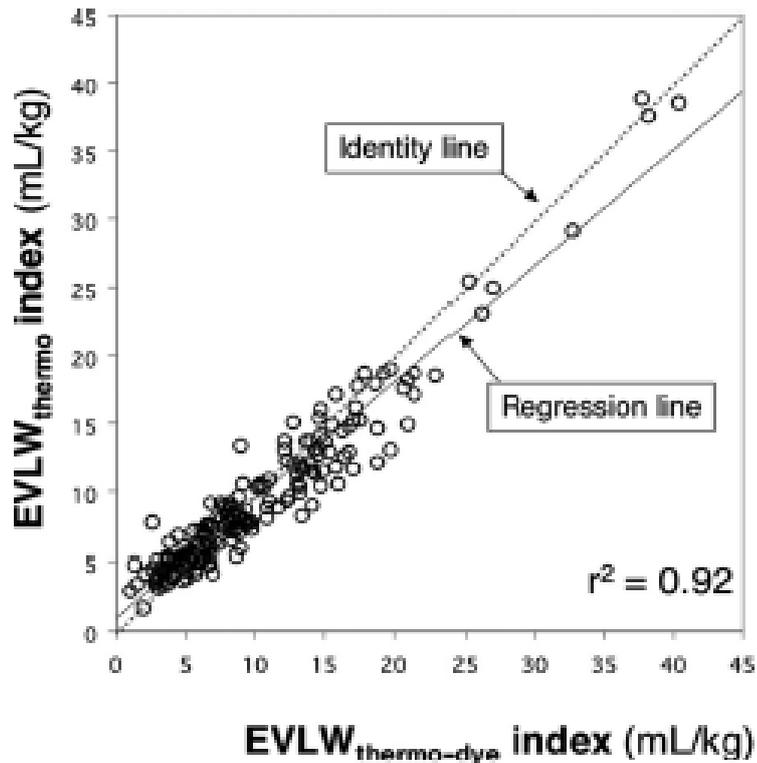


Figure 1.14 Close correlation between extravascular lung water (EVLW) estimated from the double-indicator dilution method (EVLW_{thermo-dye}) and transpulmonary thermodilution (EVLW_{thermo}) in humans (Michard, Schachtrupp et al. 2005).

The accuracy of technique is based on several assumptions. They include complete mixing with circulating blood, constant blood flow, adequate lung perfusion, no gain or loss of indicator throughout measurement, no recirculation of the indicator and constant body temperature. The thermodilution curve can be affected by the effects of respiration on venous return to the right heart, respiratory rate, timing of injection and baseline drift in the thermal signal, all which influence the calculation of cardiac output and EVLW. There are conditions that may lead to underestimation of EVLW such as pulmonary embolus, focal lung injury and lung resection. Other conditions that may give rise to inaccurate measurement are intracardiac shunts, aortic stenosis and aortic aneurysm. This technique requires regular calibration and considerable expertise to operate. It is also expensive and

invasive with the associated risk of infection and thrombosis. Nevertheless, it remains one of the most useful apparatus in the monitoring cardiopulmonary functions in critically ill patients.

1.5 Deuterium abundance analysis by Flowing Afterglow Mass Spectrometry (FA-MS)

1.5.1 Introduction

The possibility of using deuterated water to measure lung water volume was first studied in 1954. Lilienfield et al found an increase in lung water in patients with heart failure when compared with normal subjects following central intravenous injection of Deuterium Oxide (D_2O) (Lilienfield, Freis et al. 1955). However, it required multiple blood sampling and results were not available immediately. More recently, using indocyanine green and deuterium as the double indicators Wallin et al demonstrated a significantly higher lung water index in haemodialysis patients (up to 33%) than in the control group (Wallin, Jacobson et al. 1996).

Deuterium, (D or 2H), is a naturally occurring stable hydrogen isotope which is present in terrestrial water in very low concentration. The volume of body water compartment can be accurately measured following the deuterium enrichment process by isotopic dilution technique. The deuterium abundance can be determined conventionally in body fluids such as urine, saliva and blood samples by first equilibrating the test sample with gaseous hydrogen in the presence of a catalyst. The gas mixture in the headspace above the body fluids can then be sampled and the isotopic composition of this gas can be analysed by the conventional mass spectrometry, sometimes coupled with gas chromatography (Karasek F

1988). However, this approach is complex and time consuming, requiring laborious sample preparation and transportation to reference laboratories for further analysis. Hence, considerable delay can be expected from sample acquisition and analytical results to be available for clinical interpretation.

The advantage of an alternative method that enables immediate measurement at bedside that is patient friendly and does not require further complex analysis is obvious. To accomplish this, a novel ion flow tube device called flowing afterglow mass spectrometry (FA-MS) was developed. Through this approach, the HDO concentration can be determined in breath with abundant source of water vapour that can be accessed easily, or in the equilibrated headspace of any body fluid such as blood stored in a sealed container. This was made possible following the study of the determination of trace gas metabolites in breath using the selected ion flow tube mass spectrometry (SIFT-MS) (Smith and Spanel 1996). When the hydronium ions, H_3O^+ were introduced into helium carrier gas containing water vapour, hydrated hydronium cluster ions at various mass-to-charge ratio (m/z) were formed (m/z values of 73,74 and 75 are of particular interest). Such observation has led to the development of FA-MS which involves the production of thermalised hydrated hydronium cluster ions in the inert helium gas along a flow tube following the introduction of a humid air sample. These cluster ions collide with water molecules and when the isotopic compositions reach equilibrium the relative magnitudes of the isotopomers can be measured by a mass spectrometer located downstream, enabling the measurement of deuterium abundance in the air/water vapour sample.

1.5.2 Validation in healthy controls

The measurement of TBW by FA-MS has been compared with other bedside estimates of TBW in a cohort of 24 healthy subjects, 12 men and 12 women, with age distribution similar to an adult dialysis population (28-79 years)(Smith, Engel et al. 2002). TBW measurements by all methods were highly correlated ($r=0.95-0.98$, $P<0.001$). However, systematic bias was observed especially FA-MS was compared with BIA which tended to underestimate TBW in smaller female subjects. The same cohort was re-examined again after 12 months to evaluate longitudinal TBW changes (Engel, Spanel et al. 2004). High correlations between weight and TBW estimated by FA-MS, BIA and skin fold thickness (SFT) at baseline and one year later were observed ($R^2 = 0.96-0.98$) and systematic biases were effectively identical. Body composition in this group of healthy individuals was remarkably stable with the tendency of weight gain, on average 1.2 kg (non-significant) although proportion of TBWD/weight was not different.

1.5.3 Application to the dialysis patients

1.5.3.1 PD patients

FA-MS allows multiple samples to be taken in rapid succession and this enables the kinetics of D equilibration to be studied and characterised. This has been applied in the study of water transport across peritoneal membrane in patients treated with continuous ambulatory peritoneal dialysis (CAPD). An artificial deuterated water gradient was created following oral ingestion of D₂O and a typical full equilibration took place within 90 minutes. Frequent dialysate samples were taken to determine the abundance of deuterium analysed by FA-MS over time to measure water transport across the peritoneal membrane (Asghar, Diskin et al. 2003). In a further study using different hypertonic solutions (1.36%,

3.86% and extraneal) with increasing osmotic properties, the diffusive and convective components of small solute transport, including water, were characterised and as predicted by the 3-pore model of peritoneal membrane function (Asghar, Diskin et al. 2005). The headspace analysis of deuterium abundance in the dialysate fluid from this experiment provides the alternative in assessing TBW in patients following oral ingestion of D₂O.

1.5.3.2 HD patients

A typical hospital based haemodialysis treatment can last up to 12 hours per week and considerable more when travelling and waiting time is taken into account. Any further delay incurred by evaluation of body composition and fluid status is considered undesirable. Therefore, the application of FA-MS to determine TBW in haemodialysis patients requires some modification to minimise potential inconvenience. In a study to examine the validity of this technique, a protocol was devised where HD patients were given D₂O after the completion of their HD treatment and had their breath sample taken to determine deuterium abundance just prior to their next dialysis treatment 2 days later (Chan, Smith et al. 2008). The weight gain between these two treatment sessions was showed to match the water intake that caused dilution of deuterium abundance in the breath samples. Further deuterium dilution by urine output and insensible loss from sweat and breath was close to prediction, estimated to be 400ml per day. Such schedule enables the accurate measurement of TBW in HD patients without further aggravating their treatment burden.

Having established the validity and acceptability of this technique in HD population, its clinical value was further evaluated in prospective cohort study with body composition

measures at baseline and 12 months later (Chan, McIntyre et al. 2009). At baseline a strong association was found between body composition and the number of comorbidities (e.g. diabetes, ischaemic heart disease, peripheral vascular disease, heart failure etc). Patients without any comorbidity had the most normal body composition as determined by BIA, expressed as the percentage lean body mass (%LBM) in relation to body weight. As number of comorbidities increased, %LBM decreased but TBW_D actually increased, reflecting overall increase in tissue hydration despite the reduction in muscle mass. Such discrepancies by comorbidity status became more exaggerated over the 12-month follow up without any change in body weight (Table 1.2) (Engel, Spanel et al. 2004, Chan, McIntyre et al. 2009). This was especially true for the most comorbid group who actually experienced weight loss while becoming more overhydrated. Therefore the imperceptible changes of body composition can be identified by combining relative (BIA) and absolute (deuterium dilution by FA-MS) measurement of TBW.

Table 1.2 Longitudinal changes in weight, percentage lean body mass (%LBM_{BIA}) derived from BIA and TBW_D from FA-MS breath deuterium abundance following D₂O ingestion in healthy subjects and HD patients.

		Baseline	12 months	P value
Healthy Controls (n=24)	Weight (kg)	75.4	76.6	NS
	%LBM _{BIA}	64.2	64.4	NS
	TBW _D (L)	38.7	39.5	NS
	TBW _D /Wt%	51.3	52.0	NS
HD patients: Comorbidity Score=0 (n=13)	Weight (kg)	82.9	82.8	0.99
	%LBM _{BIA}	54.9	56.2	0.048
	TBW _D (L)	44.2	47.3	0.001
	TBW _D /Wt%	53.5	57.6	0.006
HD patients: Comorbidity Score 1 to 2 (n=22)	Weight (kg)	78.7	78.4	0.57
	%LBM _{BIA}	48.7	48.8	0.93
	TBW _D (L)	39.7	41.8	0.001
	TBW _D /Wt%	51.6	54.2	0.004
HD patients: Comorbidity Score >2 (n=7)	Weight (kg)	78.4	76.7	0.34
	%LBM _{BIA}	47.2	45.2	0.37
	TBW _D (L)	41.6	43.2	0.16
	TBW _D /Wt%	54.1	58.0	<0.05

1.5.4 Estimation of lung water volume using FA-MS

Following the validation of breath deuterium analysis by FA-MS in the measurement of TBW, we now consider the possibility of the application of this technique in lung water measurement. Pulmonary oedema is a potentially life threatening spectrum of

overhydration and current techniques to measure lung water excess are semi-invasive and more suited for critically ill patients in intensive care unit, rather than the relatively stable dialysis population who receives treatment in the out patient setting. We want to study the deuterium dispersal kinetic from breath analysis following the administration of a known amount of deuterated water via two routes. It may be possible to estimate the volume of exchangeable water within the airway following mouth inhalation of vapourised deuterated water. We also hypothesize following central venous injection of deuterium into the right heart, HDO rapidly equilibrates with water in the pulmonary interstitium and alveolar space during the first pass pulmonary circulation. The frequent breath sampling to determine deuterium abundance in successive exhalation may allow the evaluation of lung water volume from the extrapolation of deuterium kinetic curve. Such study design also permits the testing of the assumption that the concentration of volatiles in breath reflects their alveolar concentration.

1.6 Summary

This chapter has considered the major causes of poor outcomes in patients treated for advanced kidney failure with dialysis from which it can be concluded that cardiovascular complications present the major problem. This is undoubtedly a consequence of their accumulated cardiovascular comorbidity but it is likely that poor management of fluid status remains a major concern. Management of this problem is hindered by the lack of well validated tools available to the clinician combined with the other complicating factors associated with cardiovascular comorbidity such as cardiac dysfunction, inflammation and hypoalbuminaemia, the latter being a particular problem in PD patients. Bioimpedance represents a potentially useful tool that could be augmented by other bedside methods if

they could be shown to work, such as isotope dilution, but these require proper clinical validation.

1.7 Research questions

The study is designed to address the following research questions:

- 1 Does plasma albumin concentration affect the assessment of fluid status and fluid distribution in PD patients? How is the fluid status affected by cardiac function? This is investigated in Chapter 3.
- 2 What is the value of bioimpedance (e.g. vector analysis) in the management of fluid status in PD patients? This is the primary objective of the trial presented in Chapter 4.
- 3 Does the use of bioimpedance to manage fluid status affect cardiac function? This is the cardiac sub-analysis of the trial in Chapter 5.
- 4 Is it possible to estimate total lung water non-invasively using breath analysis of deuterium concentration by FAMS? Study investigating this using inhale or injected deuterium are described in Chapter 6 and 7 respectively.

Chapter 2 Materials and methodology

2 Chapter 2 Materials and methodology

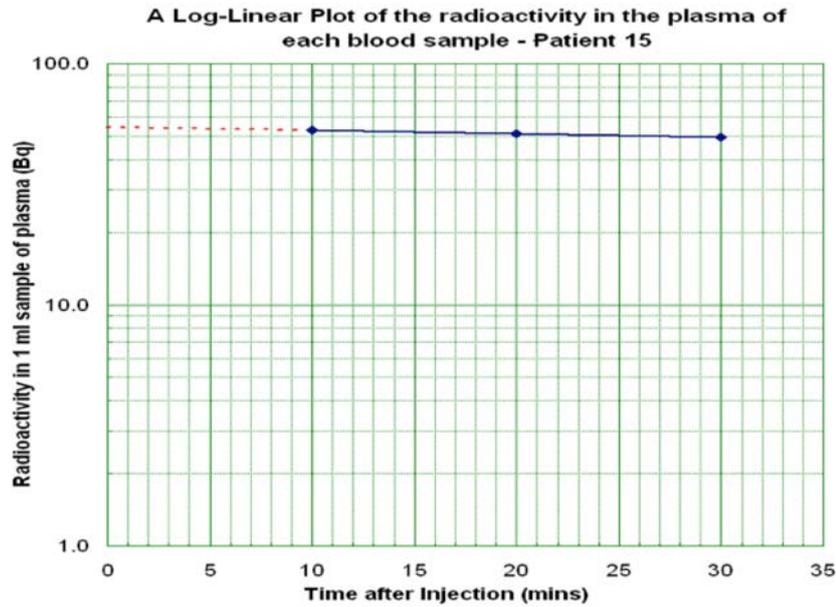
2.1 Introduction

The techniques used in this project will be described in this chapter. The measurement of plasma volume by dilution principle using human serum albumin labelled with radioactive iodine is a validated clinical technique currently available at the research site of the University Hospital of North Staffordshire. The Flowing Afterglow Mass Spectrometer (FA-MS) is a prototype built by Trans Spectra Ltd, U.K. It is a novel technique that enables the absolute quantification of deuterium concentration in exhaled breath and headspace of body fluid in real time and with minimal preparation. We collaborated with a local cardiologist with a special interest in echocardiography to measure various parameters of cardiac function described in chapter 3 and 5. The use of bioimpedance analysis (BIA) for the measurement of body composition is explored in this chapter.

2.2 Plasma volume measurement by human serum albumin labelled with radioactive iodine (^{125}I -HSA)

The use of ^{125}I -HSA to determine plasma volume has been recommended by The International Committee for Standardization in Haematology based on the technique's reliability, reproducibility and ease of operation in routine clinical use (Haematology 1980).

In this experiment a standard bolus of 10ml 0.185Mbcq ^{125}I -HSA (Schultz, Hammarsten et al. 1953) was injected into a large vein of the subject and a blood sample was drawn at a remote site at 10, 20 and 30 minute intervals. This would ensure thorough mixing within the systemic circulation. The isotopic concentration of each sample was measured in a



scintillation counter and was plotted against time on a semi-logarithmic scale. The best fit linear line was drawn through these points and extrapolated to time zero where the radioactivity at time zero could be determined (Figure 2.1). Plasma volume could be calculated by the equation:

$$\frac{\text{Radioactivity of standard } \left(\frac{\text{cpm}}{\text{ml}}\right) \times \text{dilution of standard} \times \text{volume injected}}{\text{Radioactivity of postinjection sample } \left(\frac{\text{cpm}}{\text{ml}}, \text{adjusted to time zero}\right)}$$

Plasma volume was further adjusted for gender and body surface area.

Figure 2.1 Log linear plot of the radioactivity of ^{125}I -HSA in blood sample.

2.3 Flow after glow mass spectrometry (FA-MS)

FA-MS is a new analytical approach that offers real time and on-line deuterium abundance measurements in water vapour in exhaled breath and headspace above aqueous liquids

such as blood and urine (Figure 2.2). Previous validation studies with SIFT-MS demonstrate that both accuracy and precision (reproducibility) are typically 1% for headspace sampling (Spanel and Smith 2000, Spanel and Smith 2001). Further improvement of precision of less than 1% can be achieved for breath deuterium analysis when the average value of three consecutive breath exhalations is taken.

The details of the FA-MS analytical technique have been given in previous publications (Davies, Spanel et al. 2001, Smith and Spanel 2001). Briefly, a swarm of H_3O^+ precursor ions is generated by a microwave discharge in flowing helium carrier gas possessing a trace of water vapour. These precursor ions react with the H_2O , HDO , H_2^{17}O and H_2^{18}O molecules in the water vapour contained in a breath sample (or the headspace of an aqueous liquid, e.g. serum) introduced into the helium stream resulting in the formation of hydrated clusters ions, $\text{H}_3\text{O}^+(\text{H}_2\text{O})_3$ at a mass-to-charge ratio, $m/z = 73$, and their isotopic variant ions H_8DO_4^+ and $\text{H}_9^{17}\text{OO}_3^+$ at $m/z = 74$ and $\text{H}_9^{18}\text{OO}_3^+$ at $m/z = 75$. By adopting the known fractional abundance of ^{18}O in water vapour and accounting for the contribution of the isotopic ions $\text{H}_9^{17}\text{OO}_3^+$ to the ion signal at $m/z = 74$, a measurement of the 74/75 ion signal ratio under equilibrium conditions provides the deuterium to hydrogen abundance, D/H , in the water vapour sample (R_{Ivap}). The corresponding deuterium abundance in the liquid from which the HDO is evaporating, R_{Iliq} , is derived from R_{Ivap} using a correction (coefficient factor of 0.935) that essentially accounts for the difference in evaporation rates of H_2O and HDO from the liquid to the vapour phase at body temperature.

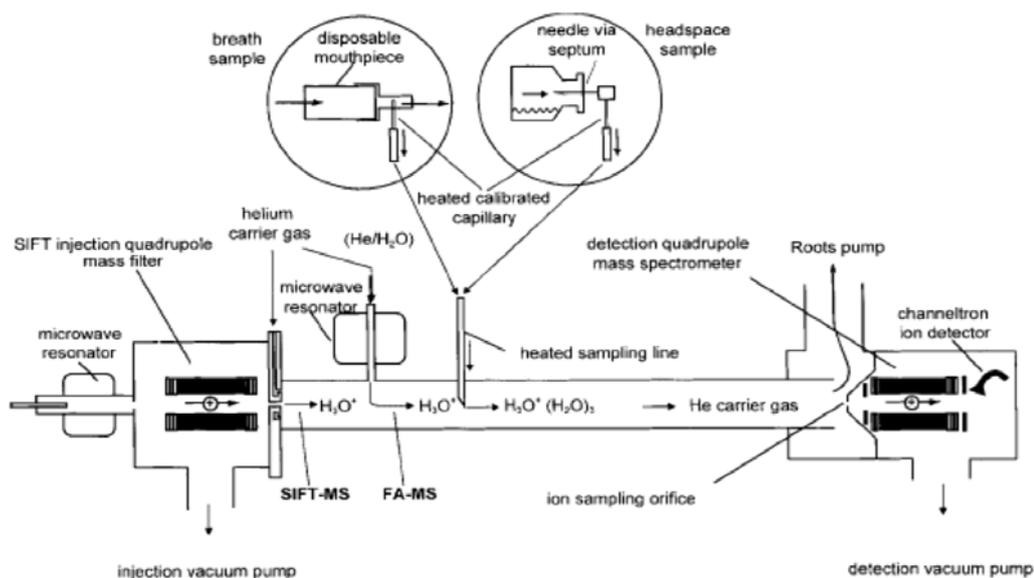


Figure 2.2 Schematic diagram of the FA-MS showing direct samplings of breath exhalation and headspace sample.

2.3.1 Direct breath sampling

Breath air is sampled directly into the flow tube via a heated calibrated capillary tube within the flow tube via a heated stainless steel tubing. The exhaled breath is delivered into the tube via a standard 15 mm diameter disposable cardboard mouthpiece which offers suitable resistance to the flow of breath such that a steady rate of exhalation can be made over a few seconds. The exhaled breath completely displaces the ambient air from the entrance of the sampling capillary and enters the coupling tubing and subsequently the flow tube/carrier gas (pressure of about 1 Torr). This is followed by oral inhalation where the entrance to the capillary is again exposed to ambient air. The flow rate is able to generate enough concentration of H₂O molecules in the carrier gas that converts of ions into the H₃O⁺(H₂O)₃, i.e. H₉O₄⁺ ions, at *m/z* 73 and its isotopomers at 74 and 75. The *m/z* 74/75 ratio signals are analysed using the FA-MS data acquisition programme (SCILIB library, version 4.96, 2005, Patrik Spänell) which provide the values of the corresponding

deuterium abundance R_{liq} . A typical time profiles of the $m/z=74$ and 75 ions are shown in Figure 2.3.

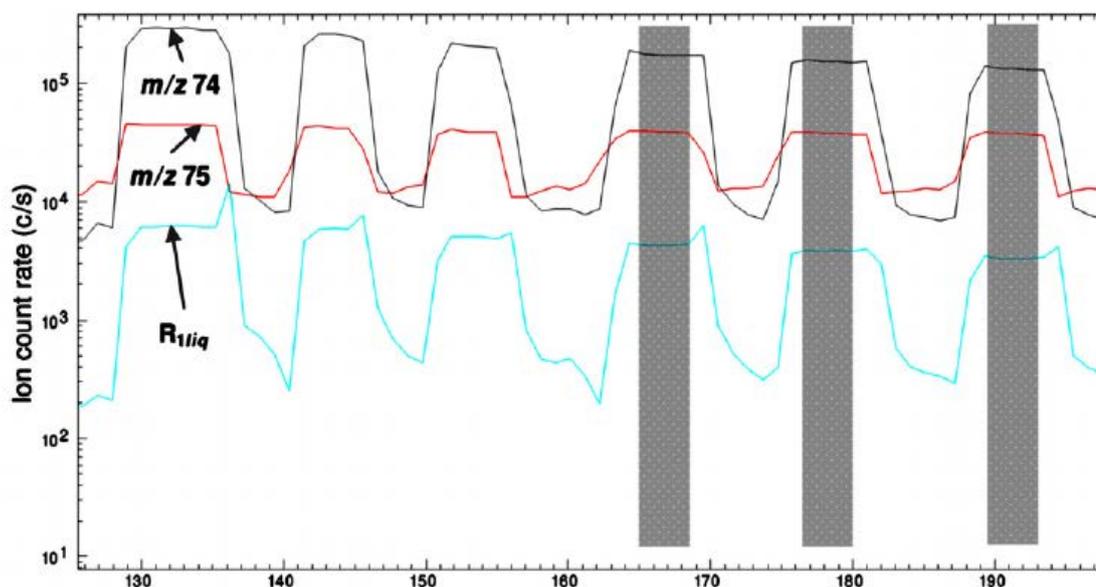


Figure 2.3 A typical time profile of breath exhalations directly sampled by FA-MS. The shaded grey areas represent the alveolar breath content.

2.3.2 Liquid headspace sampling

Deuterium (HDO) above headspace of body fluids such as blood and urine can be readily and accurately determined by FA-MS. A typical 10ml of fluid sample is placed in a 200ml glass bottle sealed tight with aluminium cap with a rubber septum in the centre. The bottle is immersed in a temperature controlled water bath, typically at around 40°C and the headspace is allowed to develop for about 10 minutes. A 23G sampling needle attached to the input line of the FA-MS instrument then punctures the septum and the vapour in the headspace is drawn into the carrier gas. The pressure in the glass bottle decreases slowly

during sampling and returns to baseline once the needle is withdrawn from the bottle. The sampling sequence consists of the alternating analysis of fluid sample and tap water to ensure stability of measurement of the instrument which is typically around 156 ppm to 164 ppm. It is critical that the sampling lines are maintained at about 100 °C to prevent any condensation of water vapour.

2.4 Bioimpedance analysis (BIA)

Multifrequency BIA was determined by using the Xitron Hydra device (Model 4200; Xitron Technologies, San Diego, CA) in the plasma volume and albumin study in Chapter 3. Before each measurement the performance and functionality of the device was tested using the electronic Verification Module (TS4201) provided by the manufacturer which simulate the typical ECW and ICW volume obtained in vivo of a male subject who is of 175 cm height and 70kg weight. The measured ECW and ICW should read between 15.81-16.18 liters and 29.39-30.74 liters respectively. Patients were required to empty the peritoneal space from all dialysate fluid prior to measurement. Patients were rested for approximately 10 mins before measurements took place according to manufacturer's manual instruction. All jewellery was removed and patients with permanent pacemaker were excluded from the study. After the skin was clean with alcohol wipes to ensure good contact, adhesive pads provided by the manufacturer were placed and connected using the standard approach of bipolar electrodes at the dorsum of the wrist and anterior aspect of the ankle on the ipsilateral side of the body (Figure 2.4). An unnoticeable alternating current was passed through the subject's body and the whole measurement took approximately 10 to 15 seconds. The resistance was measured at different frequencies at zero to infinite and finally demographic details such as age, height, weight and gender

were entered into the impedance algorithm by Xitron to generate TBW, ECW and percentage fat mass.

A different BIA device (Akern BIA 101 Anniversary, Italy) was used in the multicentre BIA study (chapter 6). It is a single frequency (50 kHz) body composition analyser which measures the resistance and reactance of the body. Measurements were taken using a standardized protocol after patients were rested for approximately 10 mins in a supine position. Patients may present with or without dialysate fluid in the peritoneal cavity but the approach was consistent throughout the study for each individual patient. Again the electrodes were placed at the bony prominences of the wrist and ankle as illustrated in the diagram below. An alternating current passed through the body and TBW and ECW were determined using a formula with good agreement with the manufacturer's algorithm. (Appendix 2)

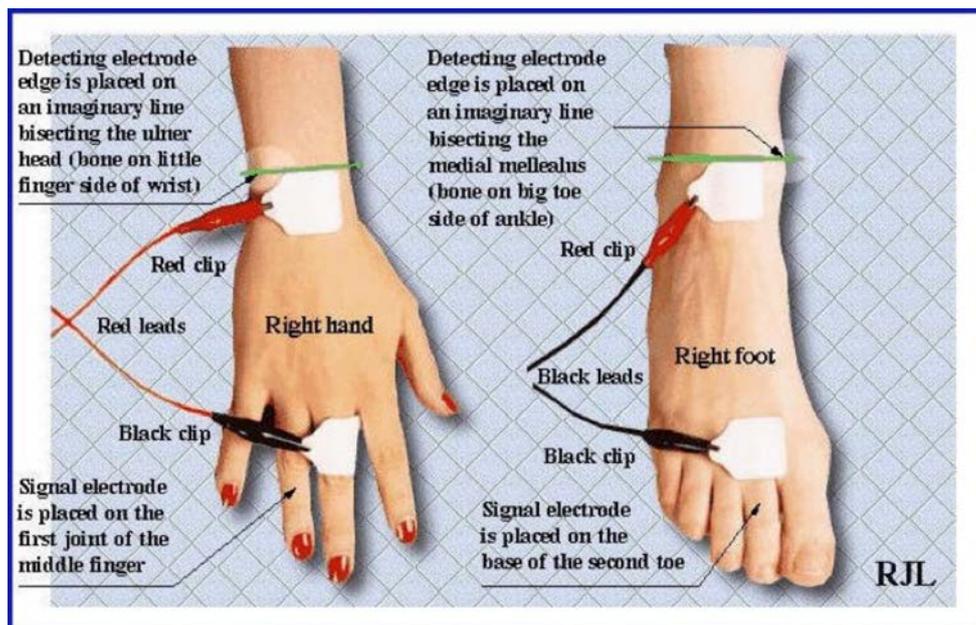


Figure 2.4 A schematic diagram showing the placement of adhesive pad and connection to the electrodes.

2.5 Echocardiography

2.5.1 Two-dimensional and tissue doppler echocardiography

All subjects underwent full echocardiography examination using a GE Vingmed Vivid Seven scanner (Horton, Norway) at rest. At least three sets of images with loops consisting of at least three consecutive cardiac cycles each were stored for offline analysis using a customized software package (EchoPac, GE). Left ventricular (LV) volume and ejection fraction (EF) were measured using the modified biplane Simpson's method from the apical 4 and 2 chamber views (Lang, Bierig et al. 2005). Left ventricular mass was calculated according to the Devereux formula (Devereux, Alonso et al. 1986) and indexed to body surface area (LVMI). Left atrial (LA) volume was calculated using the biplane area-length method from the apical 4 and 2 chamber views and indexed to body surface area to derive LA volume index (LAVI) (Lim, Ashrafian et al. 2006). The early filling (E) and atrial filling (A) peak velocities, E/A ratio, deceleration time (DT) of early filling and isovolumic relaxation time (IVRT) were measured from transmitral flow.

The mitral flow propagation velocity (V_p), an index of early diastolic ventricular suction, was measured by the slope along the aliasing iso-velocity line in Color coded M-mode images of the mitral inflow as previously described (Garcia, Palac et al. 1999, Greenberg, Vandervoort et al. 2001).

Peak mitral annular myocardial velocity of two walls of the left ventricle (septal and lateral) was recorded with real time pulsed wave tissue Doppler method as previously described (Oki, Tabata et al. 1997). The sample volume and gain were optimized, and the

Nyquist limit set to 15-20 cm/s. The early diastolic (E') mitral annular velocities were measured and E/E' was calculated (Nagueh, Middleton et al. 1997).

Color M-mode Doppler was obtained by positioning the scan line through the mitral valve with the Nyquist limit and the color baseline adjusted to obtain the best spatial resolution. Color coded tissue Doppler images were acquired over three consecutive cardiac cycles for four myocardial walls (septal, lateral, inferior and anterior) by placing a 4×4 mm region of interest in the midmyocardial area of each wall. Systolic (Sm) and diastolic (Em and Am) myocardial velocities were measured and analyzed offline as previously described (Wang, Yip et al. 2003, Wang, Yip et al. 2005). Values of all four walls were averaged.

2.5.2 Derived parameters

Stroke volume was calculated by using the aortic valve pulsed wave Doppler method whereby the velocity time integral of aortic annular flow was obtained by tracing the pulsed Doppler profile and multiplied by the area of the aortic annulus as previously described (Sun, Pu et al. 1997). Chamber stiffness was calculated by using the following equation: $70/(\text{Deceleration time}-20\text{ms})^2$ (Little, Ohno et al. 1995).

2.5.3 Speckle tracking

Left ventricular longitudinal strain, and basal and apical rotation were assessed using the speckle tracking method (Helle-Valle, Crosby et al. 2005, Becker, Hoffmann et al. 2006). Offline analysis of apical 4 and 2 chamber images, and short axis images at two levels (basal and apical) were completed by tracing the endocardium in end-diastole and the

thickness of the region of interest adjusted to include the entire myocardium. The software automatically tracks the myocardial motion on the subsequent frame and results were displayed graphically.

For global longitudinal strain data of three cycles of all six segments of 4 chamber (basal septal, mid septal, apical septal, apical lateral, mid lateral, basal lateral) and 2 chamber pictures (basal inferior, mid inferior, apical inferior, apical anterior, mid anterior, basal anterior) were averaged.

Apical rotation was calculated by averaging all six segments (septal, antero septal, anterior, lateral, inferior, posterior) of three cycles of the apical short axis view. Basal rotation was assessed by doing the same at the basal level of the short axis view. All segments were exported via customized software (XlXtrFun) into an Excel (Version 2003) spread sheet that automatically interpolated all cycles regarding to cycle length and R-waves. The software calculated twist (Apical rotation – basal rotation) and peak untwist rate (early diastolic peak of the first derivative of twist over time) (Excel spread sheet developed by A.N. Borg and S.G. Ray, University of Manchester) (Borg, Harrison et al. 2008).

2.6 Deuterium Oxide (D₂O)

2.6.1 Oral ingestion of D₂O

D₂O is a stable hydrogen isotope of water present naturally in tap water. The sterile D₂O of 99.8% purity used in this project was procured from CK Gas limited (Cambridge Isotope Laboratory, Massachusetts, United States). It was subsequently divided into

smaller vials to the standard of water for oral ingestion at the sterile fluids manufacturing units of University Hospital of Birmingham.

The preparation of D₂O for the determination of TBW by FA-MS in Chapter 3 was performed on the day of the study. The dose of D₂O used was 0.34g/kg and it was weighed to the nearest 0.01g using a calibrated digital scale at the research laboratory. After the study participants consumed the D₂O, equal amount of tap water was used to rinse out the container to ensure thorough ingestion of the measured dose of D₂O. A typical volume of D₂O dose according to the body weight of the study participants can be seen in Table 2.1.

Total body water can be calculated from the average of D/H (or R_{liq}) at equilibration and pre deuterium ingestion using the formula:

$$TBW_{FAMS} = \frac{VolumeD_2O}{\Delta(D/H)}$$

Volume of D₂O=0.34g per kg body weight

$\Delta(D/H)$ = average of equilibrated R_{liq} post ingestion of D₂O – average of R_{liq} pre-ingestion of D₂O.

The TBW value was then adjusted by 4% to account for non-aqueous exchange of hydrogen with body protein.

Table 2.1 Volume of D₂O dose based on body weight

Weight of Patients (kg)	Dose of D ₂ O (mls)
40 to 45	15
46 to 55	20
56 to 70	25
71 to 88	30
89 to 99	35
99 to 115	40
> 116	45

2.6.2 Intravenous injection of D₂O

D₂O of the highest purity (99.9%) was sourced from CK Gas Limited for the investigation of lung water measurement by FA-MS analysis of deuterium abundance in exhaled breath following intravenous injection of 10% dilution of D₂O in Chapter 7 . The neat sample of 99.9% purity was contained in 100ml sterile bottles which underwent rigorous chemical and microbiological testing by the manufacturer (see Appendix 7) prior to arrival in the UK. A local NHS facility (Stockport Pharmaceutical, Pharmacy Department, Stepping Hill Hospital, Stockport, UK) was contracted to further verify its chemical composition and microbiological safety against the European Pharmacopoeia 6.3 monographs for Water For

Injections (WFI) ((COE) 2008). The sample was then diluted with sterile water to make 10% D₂O mixture and aliquoted into 10ml vials before sterilisation.

Chapter 3 Fluid distribution in PD patients: associations with serum albumin and cardiac function.

3 Chapter 3 Fluid distribution in PD patients: associations with serum albumin and cardiac function.

3.1 Introduction

Achieving euvolaemia is difficult in significant proportion of PD patients according to evidence from many studies. Previous observational studies have showed a relationship between low salt and water removal and poor survival, independent of residual renal function (Brown, Davies et al. 2003, Paniagua, Amato et al. 2008). It is not clear, however, if this relationship is causal, with tissue overhydration or expanded plasma volume (PV) accelerating organ dysfunction or inability to optimise fluid status due to cardiac dysfunction, inflammation or malnutrition (Avila-Diaz, Ventura et al. 2006, Davies, Brown et al. 2006, Davies, Garcia Lopez et al. 2008). Individual variability in peritoneal membrane function may contribute to this problem but there is evidence to demonstrate poor ultrafiltration with high solute transport can be overcome using automated PD and icodextrin (Yang, Fang et al. 2008, Perl, Huckvale et al. 2009). This pertinent issue deserves careful consideration given the high cardiovascular morbidity and mortality in this patient population.

Bioimpedance analysis (BIA) has been widely employed in the study of body composition of dialysis patients and its data suggests that a significant proportion of PD patients are volume expanded (Plum, Schoenicke et al. 2001). The often elevated ECW/TBW ratio is extrapolated from the measured resistance and reactance using algorithms that assumes fixed tissue hydration which is not applicable for dialysis patients with deranged fluid

status. Moreover, BIA fails to distinguish between intravascular and interstitial ECW excess. This is especially relevant for PD patients whose serum albumin is often depressed from peritoneal loss or inflammation and the reduced intravascular oncotic pressure may predispose patients to fluid mal-distribution.

Structural and functional cardiac abnormalities are common and well described in dialysis population and LVH and heart failure have been showed to predict morbidity and mortality (Foley, Parfrey et al. 1995). Patients with evidence of volume expansion have more severe cardiac dysfunction which conferred worst prognosis. Heart failure with normal ejection fraction (HFNEF), previously known as diastolic heart failure, accounts for up to 80% of symptomatic heart failure PD patients and is associated with volume overload (Wang, Sanderson et al. 2003)(see Appendix 1 for HFNEF diagnostic criteria). Markers of cardiac damage and overhydration such as troponin T and cardiac natriuretic peptides have been shown to predict cardiovascular congestion and mortality (Zoccali, Mallamaci et al. 2001, Wang, Lam et al. 2006, Wang, Lam et al. 2007). Compared to HD, PD patients had higher plasma atrial natriuretic factor (ANF) and left atrial volume with more severe LVH, suggesting that elevated central venous filling contributing to overall volume expansion (Enia, Mallamaci et al. 2001). However, plasma volume was not measured in that study. There is a need to clarify the relationship between cardiac abnormalities with regards to different fluid compartments and whether PD patients are truly intravascularly expanded.

3.2 Aims of the study

1. To demonstrate that abnormal body composition observed in hypoalbuminaemic patients is associated with tissue overhydration.

2. To test the hypothesis that this measured tissue overhydration is not associated with a proportional increase in plasma volume.
3. To show that PD patients have more abnormal cardiac morphology with reduced systolic and diastolic function compared to age matched healthy controls.
4. To determine whether abnormal cardiac morphology is associated with increased intravascular rather than extravascular volume and whether PD patients with symptomatic heart failure with normal ejection fraction (HFNEF) have higher intravascular volume.

3.3 Subjects and study designs

This was a cross-sectional study of prevalent PD patients treated in a single centre with a standard clinical approach to fluid management. They include appropriate dietary advice (e.g. salt and fluid restriction), the use of diuretics to maintain urine output and use of automated PD and icodextrin to optimise fluid removal and prevent overhydration due to dialysate reabsorption.

Membrane function and dialysis adequacy are measured at least every 6 months as part of routine clinical assessment. Comorbidity is evaluated using the externally validated Stoke/Davies score (Davies, Phillips et al. 2002, Van Manen, Korevaar et al. 2003), comprises seven domains (noncutaneous malignancy, ischaemic heart disease, left ventricular dysfunction, diabetes, systemic collagen vascular disorder, peripheral vascular disease, other life threatening illness).

Patients were approached to participate in the study while attending routine clinical review. There were no exclusion criteria for the main study to evaluate the effect of hypoalbuminaemia on fluid status, providing subjects were willing to comply with the study measurements which include BIA, measurement of TBW using D dilution (TBW_D) and PV using human serum albumin labelled with radioactive iodine ($^{125}\text{I-HSA}$). Out of the 46 recruited patients, 31 patients had echocardiographic measurements and were enrolled in the cardiac sub-analysis study. They were compared with 20 healthy controls with similar age chosen from another echo study. Patients with congenital or valvular heart disease, pacemakers or implantable cardiac defibrillators, significant lung disease or anaemia were excluded from this study. The study was peer reviewed and approved by local ethics committee and all patients gave their signed consent.

3.4 Materials and methodology

BIA was determined using the multifrequency Xitron Hydra device (Model 4200; Xitron Technologies, San Diego, CA) using the standard approach as described in Chapter 2. Patients were rested for 10 mins before the measurement without any dialysate fluid in the abdomen. TBW_{BIA} , ECW and percentage fat mass were measured and compared with the normal predicted values as determined by Lindley et al for a healthy European population, adjusted for gender, weight and height (Lindley, Devine et al. 2005) and for ECW adjusted for weight and gender determined in HD patients (Chamney, Kramer et al. 2002). PV was determined according to the dilution principle using $^{125}\text{I-HAS}$.

Using deuterium dilution principle, TBW_D was measured by firstly obtaining a baseline blood sample followed by the ingestion of pre-specified dose (15ml to 45ml according to

body weight) of 99.8% deuterium oxide (see Chapter 2). After four hours an equilibrated blood deuterium was taken and the difference in D concentration in the blood headspace between the two samples was measured using FA-MS. TBW_D was determined after accounting for deuterium exchange with H in body proteins.

Routine blood measurements were made with a Beckman auto-analyzer. Plasma albumin was estimated using the Bromocresol Purple colorimetric method, (normal range 35 to 50 g/L) and C-reactive protein (CRP) using latex enhanced immunoturbidimetric method. Dialysate and urinary sodium were determined using the indirect electrode method.

Echocardiographic measurements such as left ventricular mass index (LVMI), left atrial volume index (LAVI), E/A ratio and E/E' ratio were determined as described in chapter 2.

3.5 Statistics

This is the proposed null hypothesis: The excess ECW associated with low plasma albumin is equally distributed between intravascular and extravascular spaces. The expected fluid excess associated with a plasma albumin below the median value is approximately 3L and that 20% of this (about 0.6L) should be intravascular if distributed proportionally, then to detect a significant difference ($P < 0.05$) with 90% power, assuming a standard deviation (SD) for plasma volume of 0.7L, 20 patients are required in each group.

In secondary analysis, a pre-specified univariate and multivariate linear regression analysis was undertaken treating the dependent variables (tissue overhydration and plasma volume)

and potential explanatory clinical measures (e.g. Plasma albumin, BP, comorbidity score, membrane transport status, fluid and sodium removal, log-transformed high-sensitivity CRP) as continuous covariates. If the bivariate correlation was significant related to either the dependent variable or one of the plausible explanatory clinical measures the covariate would be included in the multivariate model.

All data are expressed as means (SD) if the distribution was parametric. Non-parametric data are displayed as medians (interquartile range; IQR). Comparisons between patients were made by unpaired t test. Pearson correlation was used to study the relationship between two variables. P value of <0.05 was considered significant.

3.6 Results

3.6.1 Body composition analysis

46 PD patients (20 female) were recruited and therefore the study was adequately powered to detect the differences in the primary endpoints. The patient demography and body composition characteristics of the whole study are described in Table 3.1.

The measured ECW/TBW ratio of the patients in this study was significantly elevated compared to the normal predicted values (0.48 vs 0.45, P=0.001), indicating the possibilities of tissue overhydration, muscle wasting, or both. The measured TBW_D and the estimated TBW_{BIA} were tightly correlated but significantly different from each other such that the measured TBW_D was significantly higher, +2.02L, (95%CI: 0.96 to 3.07L, P=0.001). The almost identical relationship for the BIA data are due to the assumption of

fixed tissue hydration in all patients, whereas the TBW measured by deuterium dilution indicates variable hydration status. Most patients show evidence of overhydration. This difference, expressed as comparative plots of TBW_D and TBW_{BIA} both normalised to body weight as this relates to BIA estimated body fat mass, is demonstrated in Figure 3.1. The two measures of excess tissue hydration, i.e. the difference between measured and estimated ECW/TBW using BIA and the difference between the estimated and measured TBW by BIA and D dilution were also correlated with each other ($r=0.48$, $P=0.002$), see Figure 3.2. The latter also inversely correlated with plasma albumin (Figure 3.4).

Table 3.1 Patient demography and comparison by plasma albumin category (primary endpoint).

	All Patients (mean/% SD)		Plasma albumin <31.4 g/dL	Plasma albumin >31.4 g/dL	95% CI of difference	P value (unpaired t test)
N	46		20	26		
Gender split (M:F)	26:20		8:12	18:8	N/A	0.049
Age (years)	59.4	17.8	63.1	56.4	-17.3 to 4.0	NS
BMI (Kg/m ²)	26.5	4.9	26.0	26.8	-2.1 to 3.8	NS
BSA (m ²)	1.81	0.23	1.71	1.88	0.04 to 0.3	0.021
TBW _D (L)	36.5	7.13	35.6	37.9	-2.2 to 6.8	NS
ICW _{BIA} (L)	18.1	4.8	16.3	19.4	0.36 to 5.6	0.028
ECW _{BIA} (L)	16.6	3.5	15.7	17.1	-0.66 to 3.5	NS
Difference between measured TBW _D and estimated TBW _{BIA} (L)	2.02	3.25	3.55	0.94	0.61 to 4.6	0.012
Difference between measured and predicted ECW/TBW ratio (20)	0.029	0.035	0.036	0.023	-0.03 to 0.008	NS
Measured ECW/TBW ratio	0.48	0.04	0.495	0.472	-0.001 to -0.05	0.036
Difference between measured and predicted ECW (L/kg) (21)	0.0048	0.029	-0.003	+0.018	-0.0035 to -0.0007	0.042
Plasma Volume (ml)	2703	662	2551	2820	-124 to 661	NS
Corrected plasma volume (ml/m ²)	1474	254	1463	1482	-135 to 173	NS
Plasma Volume (% different from predicted)	-2.3	16	-0.94	-3.4	-12.2 to 7.2	NS
Corrected blood volume (ml/m ²)	2124	342	2070	2165	-110 to 300	NS
hsCRP, mean g/l, (Log CRP)	13.2 0.84	22.9 0.5	22.3 (1.05)	6.2 (0.69)	-0.64 to - 0.085	0.01

	All Patients (mean/% SD)		Plasma albumin <31.4 g/dL	Plasma albumin >31.4 g/dL	95% CI of difference	P value (unpaired t test)
Haemoglobin (g/dl)	11.7	1.34	11.1	12.3	0.52 to 1.96	0.001
Solute transport (4 hour dialysate:plasma creatinine)	0.82	0.15	0.84	0.73	-0.18 to -0.025	0.011
Peritoneal UF Capacity (ml)	426	194	408	440	-85 to 149	0.011
Proportion using icodextrin	63%	-	60%	65%	-	NS
Plasma sodium (mmol/l)	137.1	3.4	137	137.2	-1.8 to 2.2	NS
Comorbidity Score	1.00	1	1.15	1.00	-0.77 to 0.47	NS
Diabetic (%)	28%	-	25%	31%	-	NS
Systolic Heart failure (%)	6.5%	-	10%	3.8%	-	NS
Ejection Fraction (%)	56.4	8.8	56.42	56.44	-7 to 7	NS
Systolic Blood Pressure (mmHg)	137.9	20	137.6	138.1	-11 to 12.6	NS
Diastolic Blood Pressure (mmHg)	78.8	13.4	75.1	81.8	-1.1 to 14.6	NS
Peritoneal Kt/V (weekly)	1.51	0.39	1.68	1.38	-52 to -0.7	NS
Residual Renal Kt/V (weekly)	0.88	0.79	0.71	1.00	-0.18 to 0.7	0.018
Peritoneal sodium loss (mmol/day)	43.2	59.2	52.2	36.4	-51.4 to 19.7	NS
Renal sodium loss (mmol/day)	51.9	44.4	37.2	63.2	0.20 to 51.7	NS

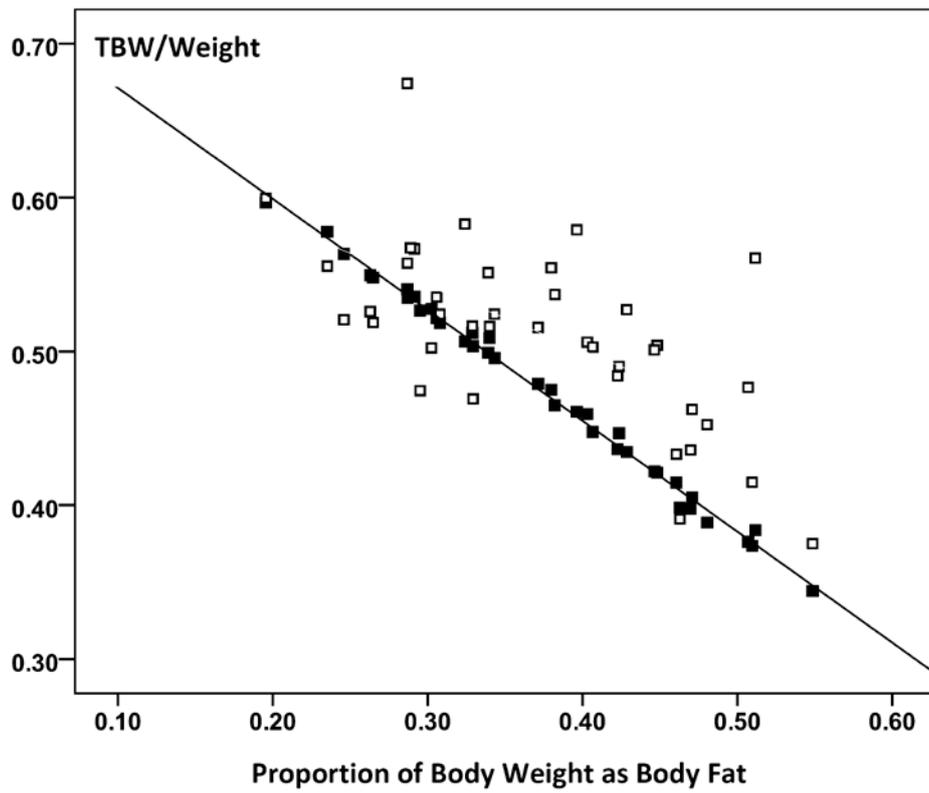


Figure 3.1 The relationship between TBW corrected for body weight determined from BIA (■) and deuterium dilution (□) and the proportion of body fat determined from BIA.

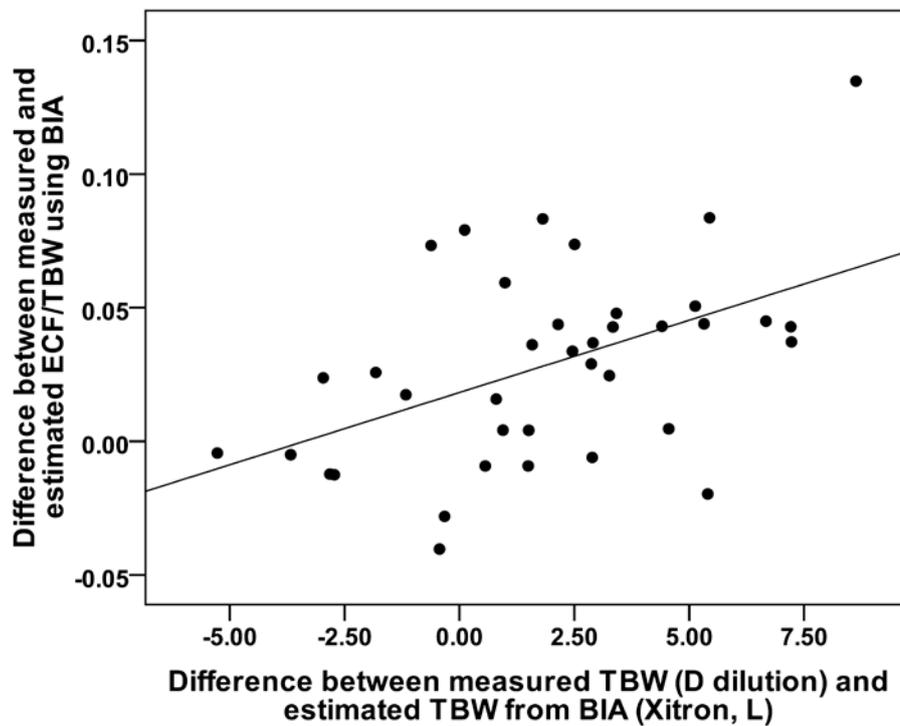


Figure 3.2 The two measures of overhydration are correlated, ($r=0.48$, $P=0.002$).

However, it was quite different for plasma volume measurement which is an indicator of intravascular volume. The measured PV was very similar to the predicted values, with a mean difference between measured and predicted of -51.3 ml (95% CI -180 to 78 ml, $P=0.428$). The standard deviation of PV was about twice that predicted indicating greater variability than expected for the normal population (Figure 3.3).

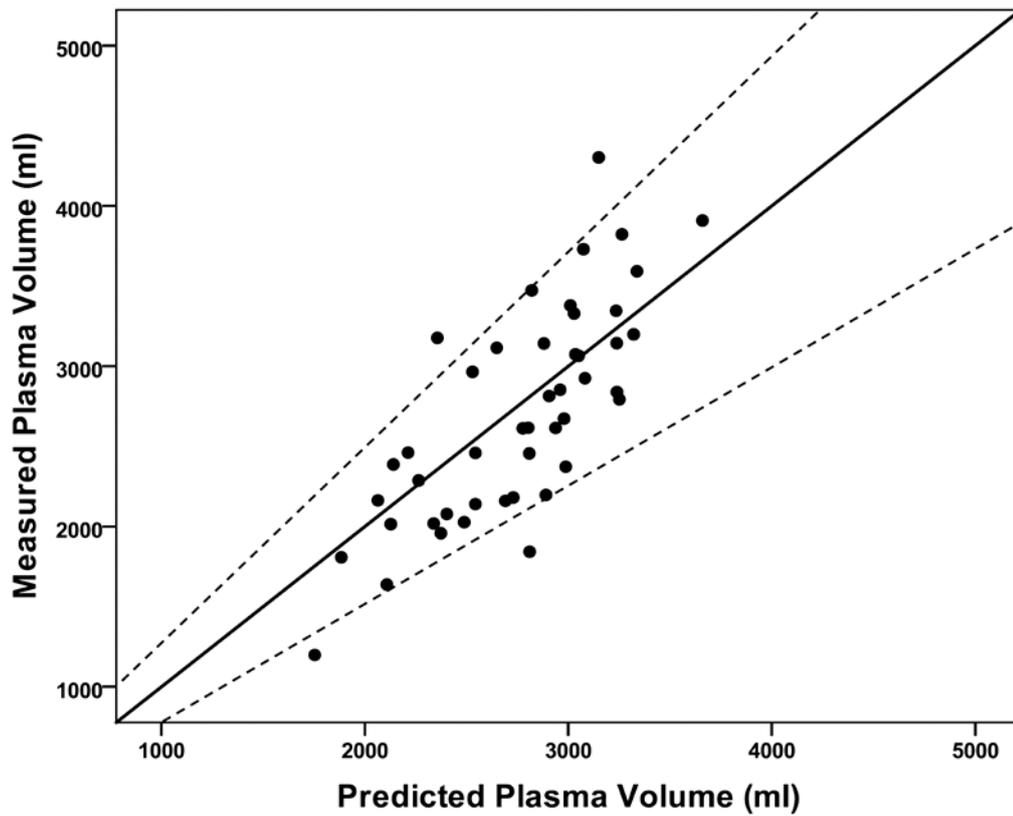


Figure 3.3 Relationship between measured and predicted plasma volume.

In order to test the primary endpoint the patients were dichotomised into those with plasma albumin above or below the median value (31.4 g/L). Participants with albumin below the median were more likely to be women, have a lower haemoglobin and with evidence of tissue overhydration and inflammation, and increased solute transport. However, the absolute and corrected plasma volumes were not different compared to subjects with no hypoalbuminaemia (see Table 3.1).

The impact on haematocrit due to the difference in haemoglobin was examined by estimating the blood volume using several different published equations corrected for body

surface area. The derived measures were not found to be significantly different between the two groups.

The summaries of the bivariate correlations for the whole study group are shown in Table 3.2. Corrected plasma volume was found to have no correlations and therefore multivariate analysis was not performed. A number of associations with excess tissue hydration were found in this analysis including inverse relationship with peritoneal sodium loss, plasma albumin and sodium, positive correlations with solute transport, peritoneal sodium loss and the use of Icodextrin. Plasma albumin was inversely related to inflammation as measured by CRP. Using the difference between estimated and measured TBW ($TBW_D - TBW_{BIA}$) as the dependent variable, plasma albumin was shown to have a negative association and it was the only relationship found to be significant in the multivariate analysis of clinical measures associated with tissue overhydration (ANOVA $P=0.011$), see Table 3.3. Replacing the ECW/TBW_{BIA} or the difference between measured and estimated ratio as the dependent variable yielded similar result. No association was found between BP and measurements of body composition and fluid status or other clinical covariates except the negative correlations between diastolic BP and both age and comorbidity. Dialysate and urinary sodium losses were closely coupled to peritoneal ultrafiltration and urine output, respectively. The incorporation of these variables in the multivariate model made no difference in the outcome of the analysis.

Table 3.2 Univariate correlation matrix

	Plasma albumin	High sensitivity CRP	Comorbidity Score	Solute transport	UF Capacity	Dialysate sodium losses	Urinary sodium losses	Using Icodextrin	Plasma sodium	Corrected plasma volume
TBW_D – TBW_{BIA}	-0.408 P=0.011	0.053 P=0.747	0.139 P=0.399	0.386 P=0.015	-0.32 P=0.048	0.323 P=0.045	-0.334 P=0.038	0.414 P=0.009	-0.393 P=0.013	0.089 P=0.588
Plasma albumin		-0.57 P<0.001	-0.139 P=0.399	-0.2 P=0.187	0.19 P=0.21	-0.077 P=0.615	0.28 P=0.059	0.092 P=0.55	0.021 P=0.89	-0.074 P=0.62
High sensitivity CRP			0.28 P=0.061	-0.016 P=0.92	-0.019 P=0.91	0.062 P=0.68	-0.02 P=0.9	-0.16 P=0.29	-0.125 P=0.41	0.19 P=0.2
Comorbidity Score				-0.068 P=0.66	-0.15 P=0.32	0.214 P=0.154	-0.27 P=0.06	0.136 P=0.37	-0.3 P=0.049	-0.215 P=0.152
Solute transport					-0.46 P=0.001	0.37 P=0.01	-0.39 P=0.008	0.53 P<0.001	-0.2 P=0.183	-0.098 P=0.52
UF Capacity						-0.19 P=0.19	0.232 P=0.12	-0.48 P=0.001	0.41 P=0.004	0.06 P=0.69
Dialysate sodium losses							-0.37 P=0.011	0.46 P=0.001	-0.224 P=0.134	0.156 P=0.29
Urinary sodium losses								0.27 P=0.07	0.22 P=0.145	0.17 P=0.25
Using Icodextrin									-0.45 P=0.002	-0.058 P=0.70
Plasma sodium										0.079 P=0.60

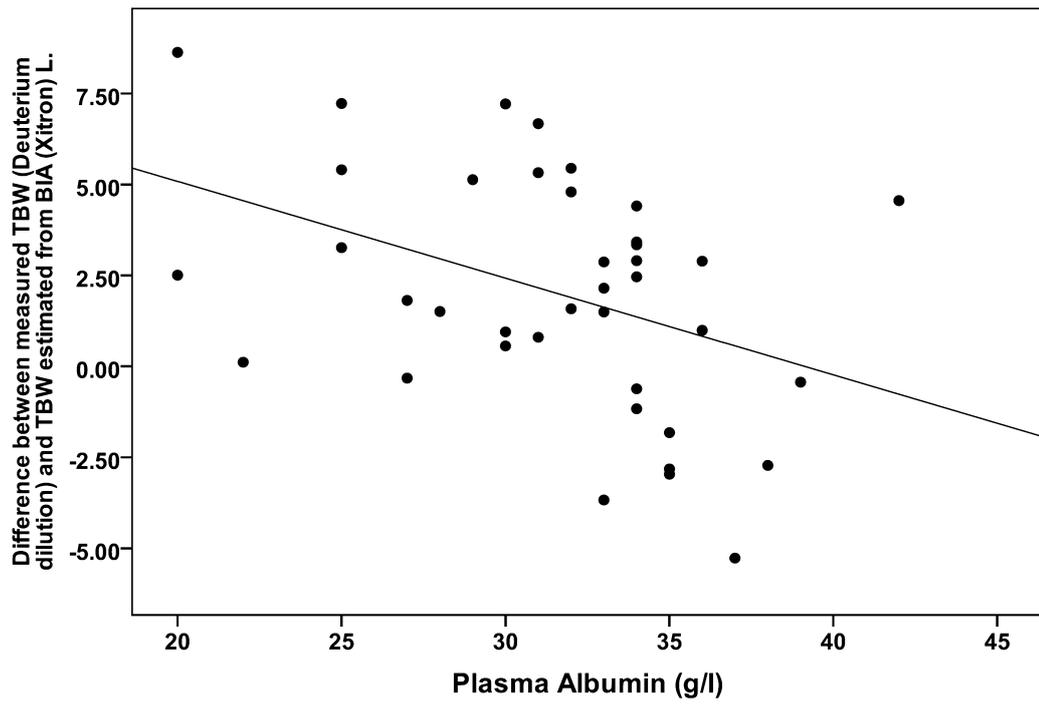


Figure 3.4 Relationship between excess fluid, as determined by the difference between measured and estimated body water, and plasma albumin ($R=-0.40$, $P=0.011$).

Table 3.3 Multivariate analysis of clinical measures associated with overhydration using the difference between estimated and measured TBW as the dependent variable.

Covariate	Standard Error	Standardised β	t	Significance
Constant	22.1		1.76	0.089
Plasma albumin (g/l)	0.12	-0.56	-3.04	0.005
hsCRP mg/l	0.025	-0.31	-1.65	0.109
Solute transport	4.1	0.09	0.50	0.62
Comorbid Score	0.51	0.096	0.60	0.55
Use of Icodextrin	1.2	0.225	1.21	0.24
Plasma sodium	0.15	-0.211	-1.32	0.197
Dialysate sodium loss	0.009	0.125	0.79	0.43
Urine sodium loss	0.012	0.049	0.29	0.77

Model Summary: $r=0.67$, ANOVA $P=0.011$.

3.6.2 Cardiac analysis

3.6.2.1 PD patients verses healthy controls

A total of 31 PD patients were included in the cardiac subgroup analysis. 20 healthy participants from another echo study matched for age and gender were used as controls.

The patients and controls demographic and clinical characteristics are summarised in Table 3.4.

Table 3.4 Demographic and clinical characteristics of patient and healthy control

	Patients (n=31)	Control (n=20)	p-value
Age (years)	60±16	63±5	0.346
Gender (female/male)	14/ 17	11/ 9	0.572
BMI (kg/m²)	26.2±4.1	24.3±3.8	0.118
NYHA	I: 3 II: 21 III: 7	N/A	
Heart rate (bpm)	72±14	71±10	0.631
Systolic blood pressure (mmHg)	140±21	139±12	0.952
Diastolic blood pressure (mmHg)	81±14	80±8	0.726
Hypertension	31/31 (100%)	0	
Years of hypertension	10.7±8.7	0	
Diabetes	8/31 (26%)	0	
Coronary artery disease	6/31 (19%)	0	
Atrial fibrillation	2/31 (6%)	0	
Duration of peritoneal dialysis (months)	25.9±18.0	0	
GFR (ml/min/m²)	8.93±5.32	78.67±12.77	<0.001
BNP (pg/ml)	96.8±99.4	/	
Corrected plasma volume (ml/m²)	1480±246	/	

BMI, body mass index; NYHA, New York Heart Association classification for heart failure; BNP, Brain natriuretic Peptide

The mean age and gender distribution between patients and controls were similar (60 ± 16 years, 45% female versus 63 ± 5 , 55% female). The medical history and antihypertensive medication were summarised in Table 3.5. 28 PD patients had symptoms of heart failure with breathlessness on exertion consistent with New York Heart Association (NYHA) class II or III. They also had normal left ventricular ejection fraction (LVEF) and left ventricular dimension. 2 patients had echocardiographic evidence of systolic heart failure with reduced LVEF of 44% and 29%. 20 patients had evidence of left ventricular hypertrophy (5 mild, 2 moderate and 13 severe) according to the criteria of the American society of Echocardiography (Lang, Bierig et al. 2005). Compared to the healthy controls, PD patients had significantly higher LVMI, LAVI, diastolic interventricular thickness and E/E' ratio (115.0 ± 31.2 vs 77.5 ± 22.7 g/m², $P<0.001$; 32.0 ± 12.5 vs 24.5 ± 8.1 ml/m², $P=0.02$; 1.2 ± 0.3 vs 0.9 ± 0.2 cm, $P=0.002$; 10.0 ± 3.3 vs 7.9 ± 2.1 , $P=0.01$ respectively) (Table 3.6).

Table 3.5 Antihypertensive medication taken by PD patients.

Class of medication	Patients
ACE-Inhibitor	13 (42%)
AT1-Blocker	6 (19%)
β -Blocker	10(32%)
Calcium-Channel-Blocker	12 (39%)
Furosemide	19 (61%)
α -Blocker	12 (39%)

ACE-Inhibitor, Angiotensin Converting Enzyme Inhibitor; AT1 Blocker, Angiotensin 1 Blocker; Calcium Channel Blocker, Calcium Channel Blocker; α -Blocker, alpha Channel Blocker

Table 3.6 Standard Echo of PD patients and healthy controls.

	Patients (n=31)	Controls (n=20)	p-value
LVEDDI (cm/m ²)	2.7±0.5	2.7±0.3	0.769
LVEDVI (ml/m ²)	46.6±15.0	43.1±13.2	0.412
Biplane LVEF (%)	57±9	61±7	0.111
FS (%)	37±9	40±7	0.160
IVSd (cm)	1.2±0.3	0.9±0.2	0.002
LVMI (g/m ²)	115.0±31.2	77.5±22.7	<0.001
LA M-Mode (cm)	4.0±0.72	3.3±0.44	0.002
LAVI (ml/m ²)	32.0±12.5	24.5±8.1	0.023
E-Wave (cm/s)	0.67±0.22	0.58±0.10	0.110
A-Wave (cm/s)	0.82±0.17	0.70±0.13	0.009
E/A	0.82±0.3	0.86±0.2	0.597
DT (ms)	237.8±66.8	256.3±50.5	0.296
IVRT (ms)	98.7±23.7	95.1±20.0	0.581
Chamber Stiffness (1/ms ²)	2.02±1.59	1.43±0.7	0.125
E/E'	10.0±3.3	7.9±2.1	0.017

LVEDDI, left ventricular end-diastolic diameter indexed to body surface area; LVEDVI, left ventricular end diastolic volume indexed to body surface area; Biplane LVEF, left ventricular ejection fraction; FS, fractional shortening; IVSd, diastolic interventricular septal thickness; LVMI, left ventricular mass index; LAVI, left atrial volume index; E-Wave, early mitral diastolic inflow velocity; A-Wave, late mitral diastolic inflow velocity; E/A, ratio of early to late mitral inflow velocities; DT, deceleration time of peak early Doppler mitral filling velocity; IVRT, isovolumic relaxation time; E/E', ratio of early mitral diastolic inflow velocity to early diastolic mitral annular velocity.

PD patients had significantly reduced mitral annular velocity both in systole (Sm)(5.0±1.2 vs 5.8±0.9 cm/s, P<0.05) and diastole (Em and Am)(4.3±1.6 vs 5.4±0.9 cm/s, P=0.017; 6.5±1.4 vs 8.1±1.6, P=0.002 respectively). In 2D speckle tracking imaging, global longitudinal strain (-18.3±3.0 vs -21.3±3.3, P=0.003) and rotation of both apical and basal levels of the left ventricular during systole (apical rotation 9.8±3.7 vs 12.2±3.1, P=0.03; basal rotation -7.0±4.2 vs -10.2±3.5, P=0.03) were significantly impaired indicating severe reduction in global systolic and diastolic function in patients (Table 3.7).

Table 3.7 Tissue doppler and speckle tracking derived parameters between patients and controls.

	Patients (n=31)	Controls (n=20)	p-value
Stroke Volume (ml)	68±19	60±12	0.119
Cardiac output (l/min)	4.8±1.6	4.2±1.1	0.157
Peak LVOT velocity (m/s)	1.01±0.2	0.94±0.16	0.184
Sm (cm/s)	5.0±1.2	5.8±0.9	0.041
Em(cm/s)	4.3±1.6	5.4±0.9	0.017
Am(cm/s)	6.5±1.4	8.1±1.6	0.002
Global Long Strain (%)	-18.3±3.0	-21.3±3.3	0.003
Apical Rotation (°)	9.8±3.7	12.2±3.1	0.033
Basal Rotation (°)	-7.0±4.2	-10.2±3.5	0.034
Torsion (°/cm)	2.4±0.9	3.2±0.5	0.01
Mitral Flow propagation velocity (m/s)	36.3±10.7	38.2±6.9	0.480

Peak LVOT velocity, peak blood flow velocity in the left ventricular out flow tract; Sm, systolic mitral annular myocardial tissue velocity by colour TDI; Em, early mitral annular myocardial tissue velocity by colour TDI; Am, late mitral annular myocardial tissue velocity by colour TDI

3.6.2.2 Relationship between cardiac morphology and fluid status in PD patients with and without Heart failure with normal ejection fraction (HFNEF)

We explored the relationship between cardiac morphology and dimension with regards to fluid compartments in PD patients. The baseline characteristics of the 31 PD patients were summarised in Table 3.8. In this subgroup analysis LVMI and LAVI correlated with corrected plasma volume (PVC) ($r=0.487$, $P=0.01$; $r=0.446$, $P=0.01$ respectively. See Figure 3.5 and Figure 3.6) but no correlation was found with other measurements of extracellular volume such as ECW/TBW, ECW/Height or the difference in TBW determined by D dilution and BIA predicted (Figure 3.7). LAVI also correlated with BNP ($r=0.644$, $P<0.01$)(see Figure 3.8). BNP, mean BP, E/E' and E/A showed no correlation with PVC. 17 of the 31 PD patients were found to fulfill the criteria for the diagnosis of HFNEF and they had significantly higher PVC compared to patients without HFNEF (1601 ± 320.8 ml/m² vs 1381 ± 225.3 ml/m², $P<0.05$) (Table 3.9 and Figure 3.9).

Table 3.8 Patient demographic and clinical characteristics.

Males:Females	17 (55%):14 (45%)
Age (years)	60.8±16.7
BMI (kg/m ²)	27.6±6.0
Diabetes	26%
Albumin (g/L)	31.4±4.0
C-Reactive Protein (mg/L)	11.8±23.4
PD duration (month)	24.8±19.5
CAPD:APD	11:20
Systolic BP (mmHg)	137.0±19.7
Diastolic BP (mmHg)	79.2±14.4
Urine volume (ml)	939.4±698.7

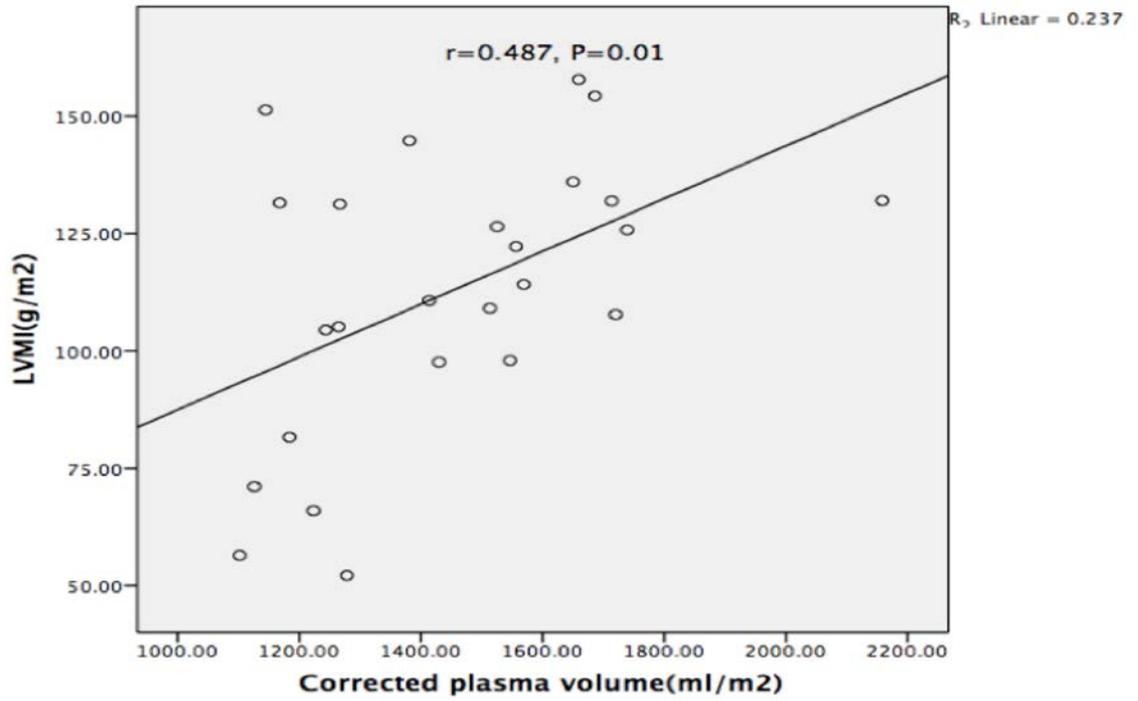


Figure 3.5 LVMI correlates with plasma volume.

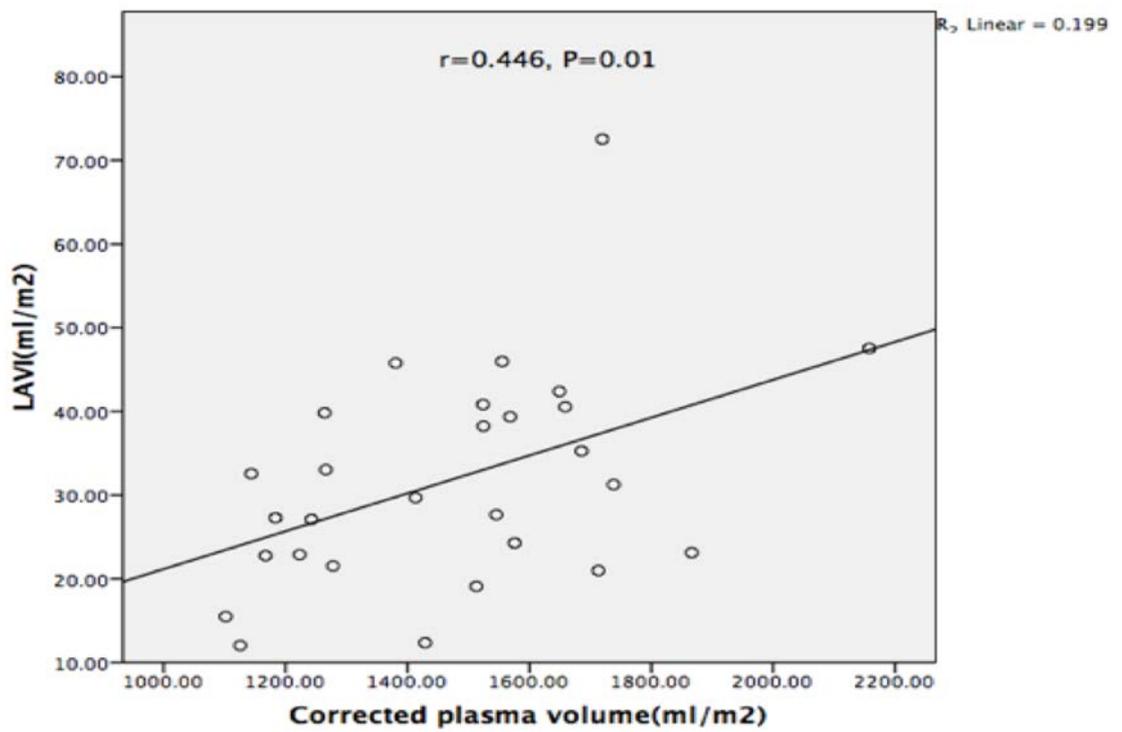


Figure 3.6 LAVI correlates with plasma volume.

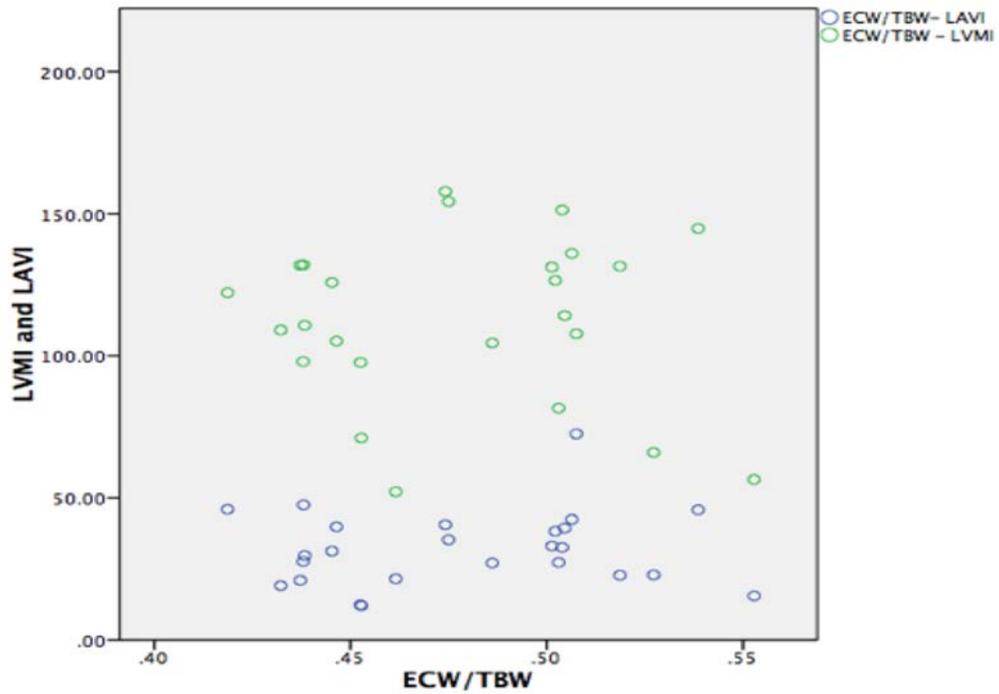


Figure 3.7 No relationship between ECW/TBW ratio determined by BIA and echographic parameters such as LVM and LAVI.

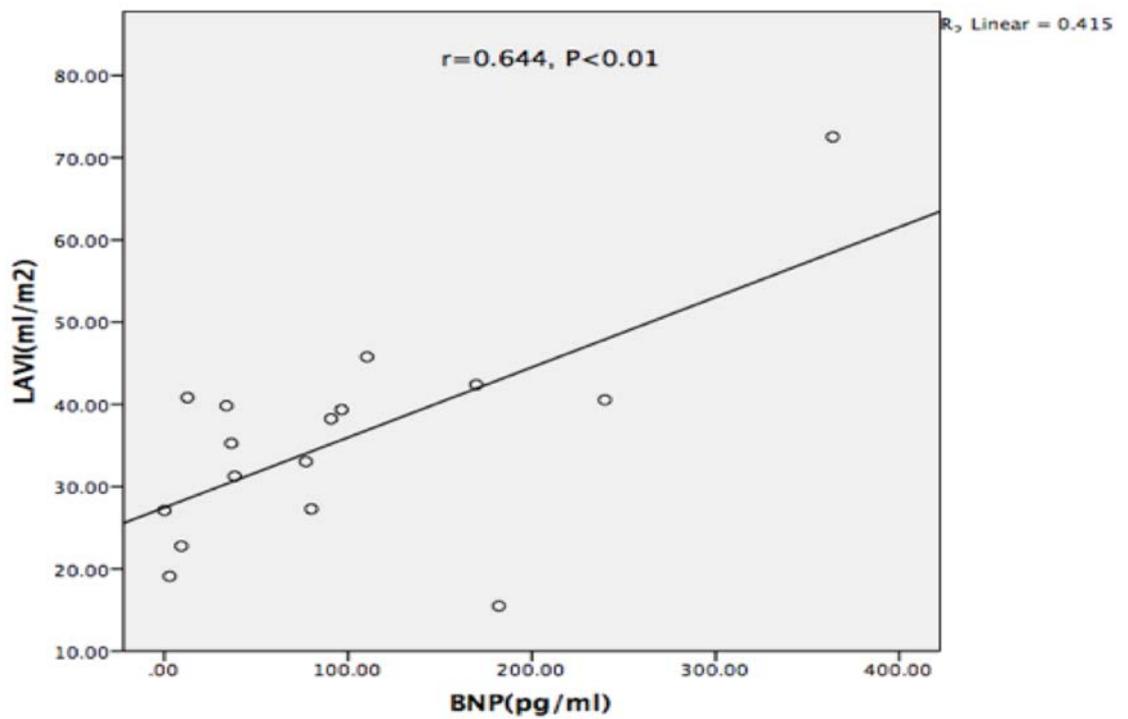


Figure 3.8 LVI correlates with serum BNP

Table 3.9 Body composition and echocardiographic measurements of PD patients with and without HFNEF (* P<0.05)

	No HFNEF (n= 14)	HFNEF (n=17)
ECF/TBW	0.48±0.04	0.48±0.03
Corrected Plasma Volume (ml/m ²)	1381±225.3*	1601±320.8
Plasma albumin (g/l)	31.3±3.1	32.7±3.5
ECF/height (L/m)	9.84±1.5	10.45±1.9
CRP (mg/l)	10.6±10.7	4.1±4.7
Age (years)	59.2±17.5	60.7±17.5
BMI (kg/m ²)	26.1±5.0	26.9±3.3
Systolic BP (mmHg)	141.7±19.1	142.2±13.7
Diastolic BP (mmHg)	82.5±15.6	79.9±13.5
LVEF (%)	55.2±10.6	58.0±7.0
LVMI (g/m ²)	102.2±34.1	113.1±33.5
LAVI (ml/m ²)	27.2±9.6	27.8±11.1
E/A	0.8±0.3	0.7±0.1
E/E	8.7±1.6	10.4±3.6

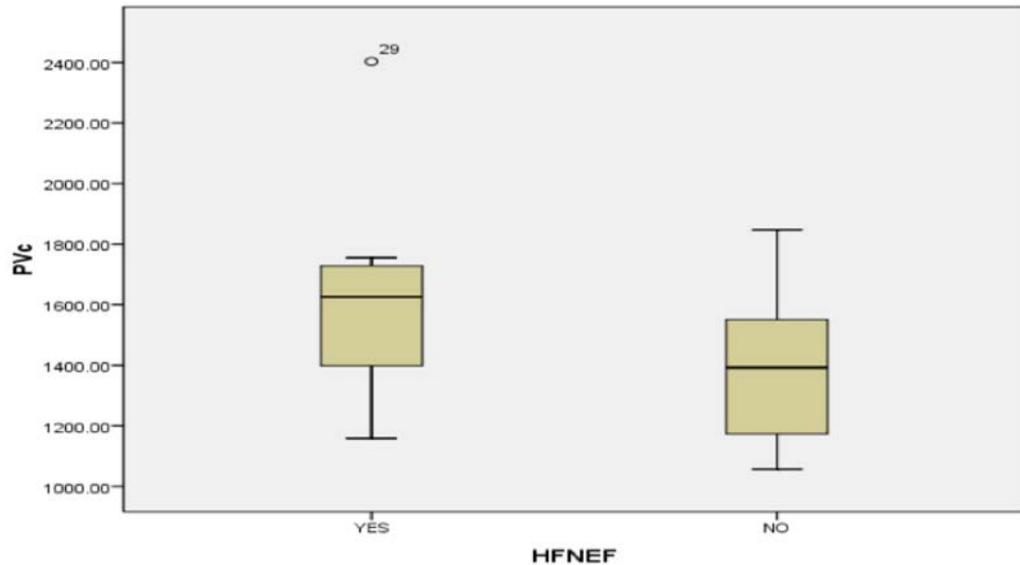


Figure 3.9 HFNEF PD patients have significantly higher corrected PV (1601 ml/m²±320.8) than non-HFNEF (1381 ml/m²±225.3); P<0.05

3.7 Discussion

This study is consistent with previous reports that ECW/TBW ratio is associated with low plasma albumin and inflammation and this relationship is found to be associated with measurable fluid excess in PD patients. In support of the study hypothesis the excess fluid is not distributed equally in the intravascular compartment and it is therefore reasonable to conclude it is predominantly found in the extravascular space. It is thought the reduced oncotic pressure secondary to hypoalbuminaemia leads to underfilling of plasma and this is supported by the multivariate analysis that plasma albumin becomes the only factor determining the hydration status in PD, overriding other clinical measures of overhydration. Plasma albumin has been demonstrated to be associated with inflammation but in the context of peritoneal dialysis it is also affected by peritoneal protein loss (Kaysen, Yeun et al. 1997). This has clinical implications as any attempts to normalise

fluid status in hypoalbuminaemic PD patients may lead to central volume depletion and loss of residual renal function.

This is the first study to measure plasma volume using ¹²⁵I-HAS dilution technique and hydration status simultaneously in PD patients. Koning et al previously measured similar parameters by extrapolating the disappearance rate of Dextran 70 and found slightly higher plasma volume of 3200ml compared to the study (2700ml) (Konings, Kooman et al. 2002) probably reflecting difference in techniques and gender split (75% male compared with 56% in this study) hence body sizes between the two groups of participants. The values were more similar once corrected for body surface area (1684 verses 1474ml). However the discrepancy in PV could be real, indicating overhydration could be more common in Konings group since the study was conducted before the use of Icodextrin and 1.36% glucose dialysate bag was routinely used for long dwell (Konings, Kooman et al. 2002).

One of the advantages of using ¹²⁵I-HAS to measure PV, previously not possible with Dextran 70 technique, is its use in patients using Icodextrin, thus providing the first reported PV measurement in PD patients using this dialysis solution. It was unclear whether patients using Icodextrin may experience excessive intravascular depletion leading to early loss of residual renal function or relatively preserve it by a given fall in ECW by ameliorating a precipitant fall in PV. Its use was associated with overhydration on univariate analysis but not to PV, gender split or any other clinical measures. This relationship, however, disappeared on multivariate analysis, underlying the complex relationship between peritoneal solute transport, ultrafiltration, plasma albumin, isotonic hyponatraemia and residual and peritoneal sodium losses in PD patients. The opposite

relationship between overhydration and urinary versus peritoneal sodium losses is intriguing. One possible explanation is better fluid status is maintained with preserved renal function which enables adequate salt and fluid removal whereas Icodextrin was selectively used in patients with higher transport status associated with reduced ultrafiltration and sodium removal (Konings, Kooman et al. 2002). The end result is that solute transport is not independently associated with overhydration. The correlation between low plasma albumin and urinary but not dialysate sodium removal and these patients lose less urinary sodium for a given residual renal clearance could be due to activation of renin-aldosterone system in the context of hypoalbuminaemia.

The inverse relationship between plasma albumin and abnormal body composition as measured by BIA has been shown in previous studies (Jones, Smye et al. 1998, Jones, Akbani et al. 2002, Avila-Diaz, Ventura et al. 2006). The elevated ratio of ECW/TBW is frequently thought to be a measure of tissue overhydration despite the fact that both raised ECW or decreased TBW, for instance in muscle wasting could be responsible. A different approach was used in this study by examining the relationship between hydration status and the difference between measured TBW_D and estimated with BIA. In the Chamney model the tissue hydration index is constant and independent of body size and fat and any measured extra fluid above this constant is considered a marker of overhydration (Chamney, Wabel et al. 2007). In Figure 3.1 the near perfect correlation for TBW measured by BIA at a given lean body mass suggests the assumption of constant tissue hydration index. Majority of PD patients are shown to be overhydrated. This is especially true in hypoalbuminaemic patients, further supporting the study's hypothesis. This provides an independent evidence that low ECW/TBW or raised ECW/weight can reflect fluid excess, also in keeping with previous study using sodium bromide dilution to measure

ECW in PD patients (Konings, Kooman et al. 2002). Using the similar approach to estimate degree of tissue overhydration in a longitudinal study in haemodialysis patients, the average fluid excess at baseline postdialysis was just a little less (1.62kg) than that observed in this study (2.02kg). Instead of plasma albumin, comorbidity was the most important clinical predictor of overhydration in HD patients (Chan, McIntyre et al. 2009). This is likely a reflection that the variance in comorbidity is greater in HD patients, whereas the spread of plasma albumin levels is greater in PD patients.

The main findings of the cardiac sub-analysis are as follows:

1. Compared to non-hypertensive age matched controls PD patients treated with standard clinical care demonstrate several cardiac abnormalities including LVH, atrial enlargement and diastolic dysfunction.
2. The plasma volume is in the normal range in more than 85% of PD patients. This seems to suggest that measured fluid excess is largely extravascular and is associated with inflammation and low albumin.
3. The cardiac structure abnormalities are associated with intravascular volume and not the measures of total fluid excess.
4. Other validated echocardiographic assessment and biomarker of filling pressure such as E/E', E/A and BNP do not correlate with plasma volume, hence may not reflect intravascular volume.
5. PD patients with symptomatic HFNEF have a higher plasma volume.

Using a cohort of age and gender matched healthy adults from another echo study in our local research centre, 93% (28/31) of the PD patients exhibited NYHA class II and III heart failure symptom with breathlessness on exertion and 62% (20/31) has varying degree of LVH. We further demonstrated that PD patients had significant cardiac structural and functional abnormalities from echocardiographic parameters which may account for the prevalent symptoms of cardiac decompensation in the absence of other explanations such as anaemia. A decrease in mitral annular velocity in systole and diastole, together with reduction in global longitudinal strain, apical and basal rotation seems to suggest a picture of 'stiffening' of the heart and global systolic and diastolic dysfunction. However, only two patients (7%) showed signs of systolic heart failure with reduced LVEF of 44% and 29% respectively while majority of the patients (94%) have normal or near LVEF, indicating preserved systolic function. This finding is consistent with Wang et al where only 8% of PD patients had systolic dysfunction as defined by EF of <45% whereas 80% had diastolic dysfunction (Wang, Sanderson et al. 2003).

PD patients are commonly perceived to be volume expanded in both intravascular and extravascular compartments but there has been a relative paucity in the study of fluid compartments in this cohort of patients. Measurement derived from BIA is not able to discriminate between different compartments but most body composition studies have employed this technique given its easy application and clinical validation with other gold standards particularly in subjects with normal fluid status. Surrogate biomarkers such as atrial natriuretic peptides (ANP) have been found to correlate central venous filling or pressure. Plum et al showed despite having a higher ECW/TBW ratio than patients before haemodialysis treatment, the serum ANP concentration in PD patients corresponded more to post HD values (Plum, Schoenicke et al. 2001). This is supportive of the hypothesis that

volume expansion in PD patients is predominantly in the interstitial compartment with relatively normal intravascular volume. Our study has shown that the plasma volume is within the normal range in over 85% of PD patients and it is therefore reasonable to conclude that measured fluid excess is chiefly in the extravascular space.

We showed for the first time in this patient group who had predominantly diastolic not systolic dysfunction that standard echo parameters such as LVMI and LAVI were related to plasma volume but not other measures of fluid excess such as ECW/TBW, ECW/height or the difference in the BIA predicted and deuterium determined TBW. It is not clear if deterioration in left ventricular function leads to plasma volume expansion or vice versa. Future interventional studies designed to improve these cardiac echo parameters by rendering patients hypovolaemic would be needed to determine this. However this may compromise residual renal function which may not be desirable.

BNP is a cardiac natriuretic peptide and its release is dependent upon ventricular myocyte response to stretch with increase in filling pressure. It is universally raised in ESRD population on dialysis therapy and the main reasons include high incidence of LVH and ventricular dysfunction and reduced renal clearance (Buckley, Sethi et al. 1992). It detects cardiac ischaemia and heart failure and predicts prognosis of heart failure (Davis, Espiner et al. 1994, Yu and Sanderson 1999, Berger, Huelsman et al. 2002). BNP has been shown to correlate with marker of LV filling pressure (E/E') (Mak, DeMaria et al. 2004) and fall in parallel with reduction in pulmonary capillary wedge pressure following treatment of acute heart failure (Kazanegra, Cheng et al. 2001). This seems to suggest the potential of BNP as a surrogate marker of fluid status. Finally, BNP has been shown to correlate with

LVMI and LVEF and predict cardiovascular congestion, overall mortality and cardiovascular death (Zoccali, Mallamaci et al. 2001, Wang, Lam et al. 2007). However, we did not find any relationship between BNP and measures of fluid compartments such as plasma volume or ECW/TBW. The association between BNP and LAVI but not LVMI in our study is intriguing. LAVI reflects LA pressure and it has been shown that patients with more severe diastolic dysfunction have larger LA volume in a large cross sectional study in general population (Pritchett, Mahoney et al. 2005). This is likely to be the case in PD patients with high incidence of diastolic heart failure with raised BNP.

A large proportion of the patients in the study had breathlessness on exertion, a symptom suggestive of heart failure. Majority of them had normal ventricular ejection fraction on echographic examination. Previously known as diastolic heart failure, it is now referred to as heart failure with normal ejection fraction (HFNEF) or preserved ejection fraction. Often found in patients with history of volume overload and LVH, HFNEF is present in 80% of PD patients (Wang, Sanderson et al. 2003). This is in keeping with previous study where hypertensive pulmonary oedema was associated with diastolic and not systolic dysfunction (Gandhi, Powers et al. 2001). Diastolic dysfunction was also commonly found in patients presenting with systolic heart failure where over 90% showed varying degree of abnormal relaxation pattern and those with restrictive filling pattern had the highest cardiac mortality (Yu, Sanderson et al. 1996). Our study has indicated for the first time PD patients who fulfilled the HFNEF criteria have significantly higher plasma volume compared with those who do not. It is not clear if the raised plasma volume is secondary to or the cause of HFNEF. It could also be argued that higher intravascular volume is required to enable adequate filling into a stiff ventricle. Further clarification in future study is needed to address this question.

As already discussed the cross sectional nature of this study is its main limitation allowing associations rather than causal inferences to be made. It can be argued that the cause of low albumin is a consequence of intravascular dilution rather than a cause of extravascular fluid accumulation but one would expect higher PV which is not the case in this study. However, no correlation was found between PV and plasma albumin or its rate of disappearance from the vascular compartment. There was a significant spread of residual renal function in this study involving only prevalent patients out of necessity known to influence fluid status. This study with relatively small sample size contains many covariates and multiple testing may increase the probability of false positive. This can be controlled by adopting the Bonferroni correction that set the significance cut-off at the acceptable level. However, by being more conservative in the Type I error rate for each testing, the chance of missing a genuine difference in the data would increase. Another concern using multivariate regression analysis with several covariates is collinearity. This is especially true where there is high correlation amongst some of the covariates. This could increase the risk of both false positive and false negative that could cause confusion in the interpretation of results from the regression analysis. The TBW volumes were measured and estimated by deuterium dilution and BIA respectively. Both were found to be highly correlated but significantly different. The degree of agreement between the two methodologies can be assessed by the use of Bland Altman plots and any systematic difference can be identified. The predicted difference in overhydration between the pre-defined groups and SD of the PV were similar to the value used in power calculation though the degree of overhydration was less in the study. Non-significant difference in corrected PV in both groups and lower than predicted SD makes type 2 error unlikely. The unequal distribution of gender and haemoglobin between groups was taken into account in

the analysis. No association was found between BP and other measures of fluid status and this could be confounded by patients taking antihypertensive medication.

3.8 Conclusions

Hypoalbuminaemic PD patients are overhydrated. Their tissue overhydration is not associated with expanded plasma volume; hence by inference fluid excess is predominantly in the extravascular space. Tissue oedema is not correlated with the very abnormal hearts of PD patients, at least when the predominant abnormality is diastolic dysfunction. Symptomatic HFNEF may be associated with modest central volume expansion. Attempts to reduce tissue overhydration may result in further intravascular depletion and thus may have adverse impact on haemodynamic stability and residual renal function.

**Chapter 4 A trial to determine the value of bioimpedance
vector plots in the longitudinal management of patients
on peritoneal dialysis**

4 Chapter 4 A trial to determine the value of bioimpedance vector plots in the longitudinal management of patients on peritoneal dialysis

4.1 Introduction

Optimal fluid management is one of the primary objectives of dialysis treatment and there is significant concern that peritoneal dialysis (PD) patients can become progressively fluid loaded with time on treatment, especially as residual kidney function declines (Tan, Chan et al. 2010, Van Biesen, Williams et al. 2011). There is a growing body of evidence that bioimpedance (BI) analysis has a role to play in assisting the clinician in managing fluid status (Davies and Davenport 2014). This is primarily based on observational studies showing that overhydration as determined from BI, predicts worse survival (Pillon, Piccoli et al. 2004, Machek, Jirka et al. 2010, Paniagua, Ventura et al. 2010) and the demonstration that BI can detect changes -following interventions designed to improve fluid status (Davies, Woodrow et al. 2003). BI data comprises two components, resistance to an electrical current, typically passed through the body from wrist to ankle which is inversely proportional to TBW, and reactance which is the impedance to this alternating current, also measured in Ohms, as it passes through tissues with cell membranes and thus proportional to cell mass. These two components can be plotted as a two dimensional vector and used to track changes in fluid status that could support clinical decisions (Figure 4.1) However, few clinical trials have been conducted that clearly demonstrate a benefit of BI over and above good quality standard clinical management. Longitudinal studies of body composition indicate that progressive overhydration is usually associated with a decline in

muscle mass and a potential failure to adjust the dialysis prescription so as to reduce the extracellular water (ECW) volume down in parallel with this (Chertow, Johansen et al. 2000, Johansen, Kaysen et al. 2003, Chan, Smith et al. 2008, Davies, Engel et al. 2013).

4.2 Aims of the study

We hypothesised that the longitudinal application of BI alongside clinical evaluation would help the clinician identify this problem and thus make appropriate adjustments to the prescription. To test this hypothesis we undertook a randomised controlled trial to determine whether the additional information available from longitudinal BI over 12 months could assist in maintaining stable or improved fluid status. Our aim was to determine whether routine clinical management supported by the longitudinal plot of the BI vector, which shows the direction in which fluid status is changing, resulted in more stable fluid status than control subjects. The outcome, to which the clinicians were blinded, was fluid volumes, ECW, total body water (TBW) and their ratio (ECW/TBW) as determined from the BI measurements after the trial was completed.

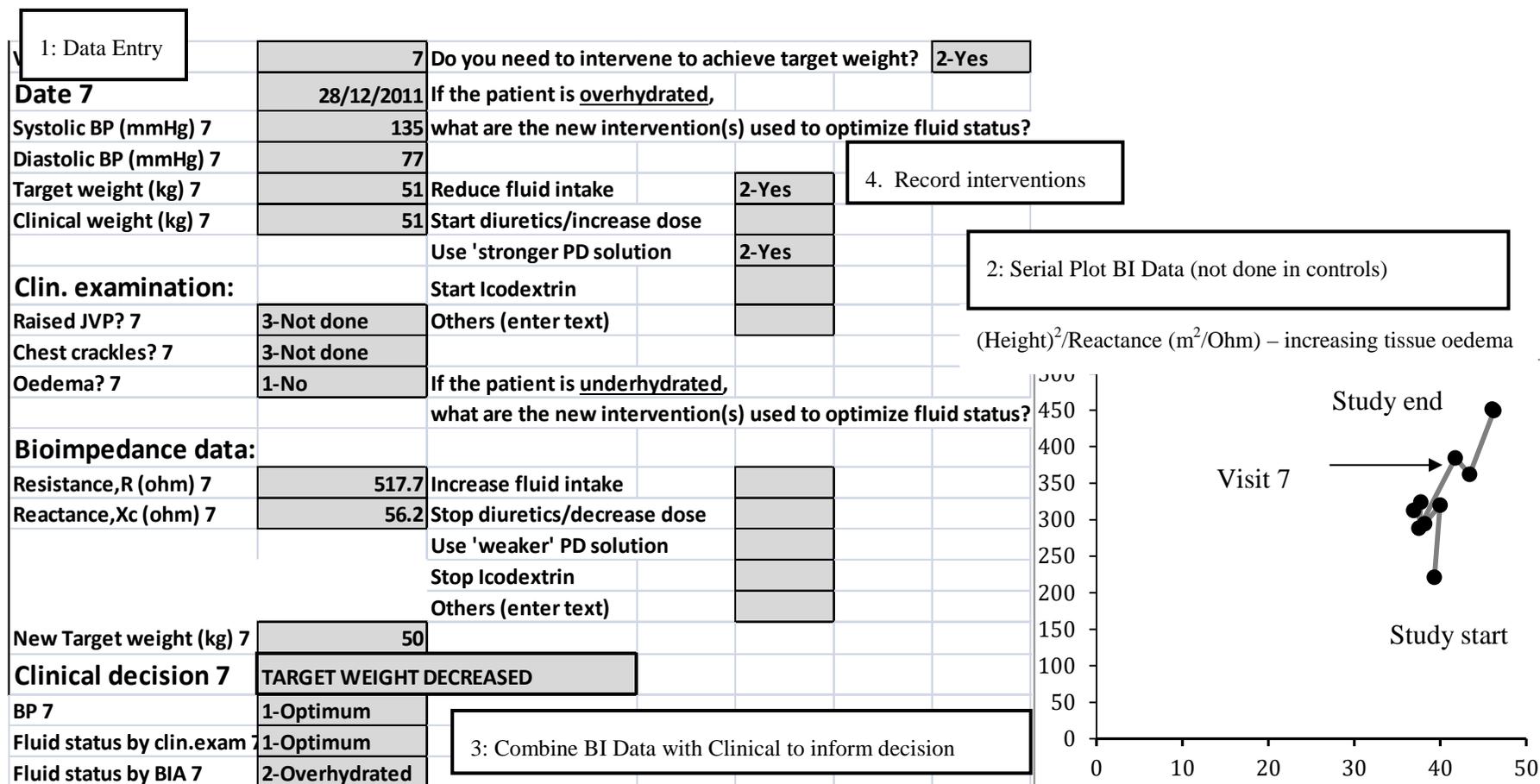


Figure 4.1 The procedure for documenting clinical interventions.

4.3 Subjects and study designs

4.3.1 Subjects

Four independent randomisation groups comprising non-anuric and anuric patients from 3 UK dialysis centres (Stoke-on-Trent, Leeds and Sheffield) and one Chinese centre (Shanghai) respectively.

4.3.2 Study Design

The study design was a pragmatic, nested, controlled trial based on the PROBE principles (Hansson, Hedner et al. 1992), i.e. prospective, randomised, open-label, blinded end-point in which patients were allocated 1:1 either to an active arm in which BI measurements were available to clinicians in their assessment of fluid status or a control arm in which measurements were taken but concealed. Complete blinding of control BI data was achieved by using vector analysis which requires two dimensional plotting (Figure 4.1 and Figure 4.2) prior to their interpretation. In addition, clinicians were blinded to the primary body composition outcome for all participants as BI derived fluid compartment volumes (ECW, TBW) were only calculated after completion of the study and data lock.

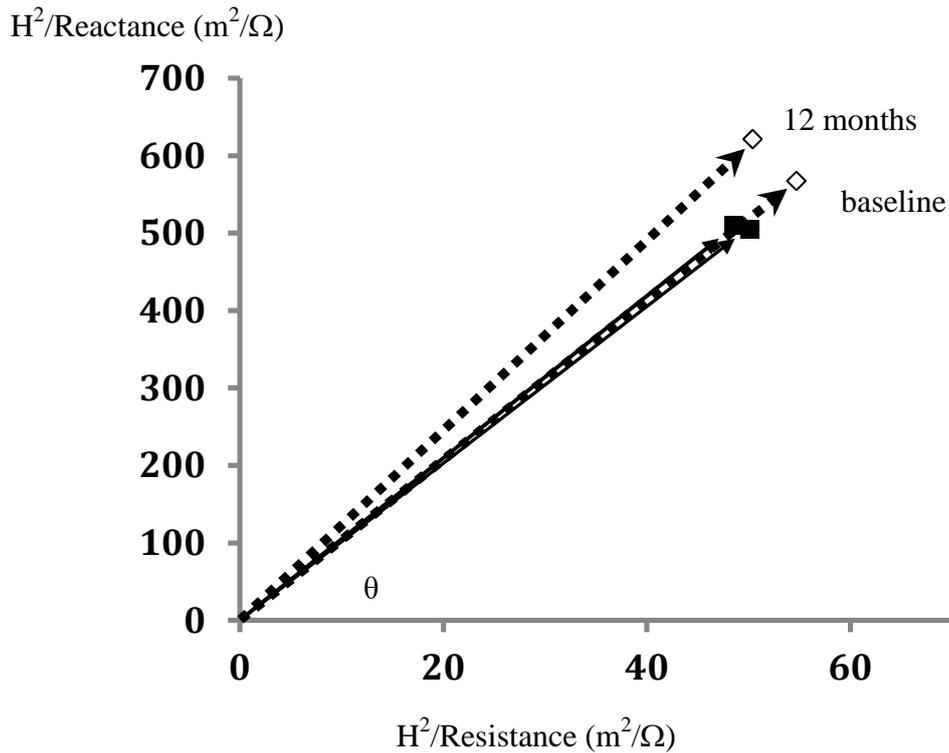


Figure 4.2 Vector plot showing mean changes in bioimpedance and their associated vectors for the Shanghai-anuric patients (Controls ◇, Active ■)

Within this overarching design, 4 groups of patients were recruited each using an independent, concealed and randomly generated centralised allocation procedure. The 4 groups were non-anuric and anuric, defined as a 24 hour urine volume <200 ml, with patients recruited from 3 medium sized UK centres, and one large Chinese Centre. The rationale underpinning the need for four independent patient groups nested within a common trial design is that (1) clinical decisions are likely to be affected by the presence of residual kidney function, (2) that the options for therapeutic intervention are different in the UK, where automated PD and icodextrin are available compared to China where options are more limited despite a higher proportion of anuric patients and (3) that there were anticipated important differences in case mix between the two countries, e.g. body weight and comorbidity.

Pragmatic inclusion and exclusion criteria were employed to mirror routine practice. Clinicians were asked to include sequential patients attending clinic who were willing to be enrolled only excluding patients unlikely be on peritoneal dialysis for more than 6 months for whatever reason. All subjects gave fully informed consent and the trial was pre-registered with ClinicalTrials.gov Number: NCT00801112 and was later included in the National Institute for Health Research Clinical Research Network portfolio (Appendix 4).

Following the obtaining of consent clinicians were allowed up to 3 months to stabilize fluid status using standard clinical assessment (but not BI) and ensure that the patients had recovered from any inter-current illness, e.g. peritonitis. Immediately following randomization fluid assessments (including BI measurements) were undertaken a minimum of three monthly over the following year unless the subject left the study. Additional assessments were permitted including use of BI in the active limb at the clinician's discretion. The primary outcome was body composition as calculated from BI. Secondary outcomes included blood pressure, residual kidney function, peritoneal membrane function (solute transport and ultrafiltration capacity) and prescribed dialysis dose.

Standard assessment of fluid status was recorded using an electronic database (Figure 4.1-step 1) that included weight, physical examination (e.g. oedema,) and blood pressure. In the BI groups a vector plot was automatically generated (Figure 4.1-step 2) and clinicians were trained prior to the study to use the longitudinal plot of the $H^2/\text{Resistance}$ (\approx Total Body Water) and the $H^2/\text{Reactance}$ (\approx Extracellular Water) to determine the significance of longitudinal changes in fluid status and change the target weight so as to keep the vector

length and angle as stable as possible (Figure 4.1-step 3). This was then combined with clinical observations to inform the decision and the methods apply to achieve this was recorded in step 4. Lengthening of the vector represents an increase in total fluid content, shortening the opposite; increase in the vector angle indicates less lean tissue, and thus more fluid in the extracellular space whereas a reduction in the vector angle implies increased lean tissue thus either reduced extracellular water or increased muscle depending on whether the vector shortened or lengthened (see Figure 4.3).

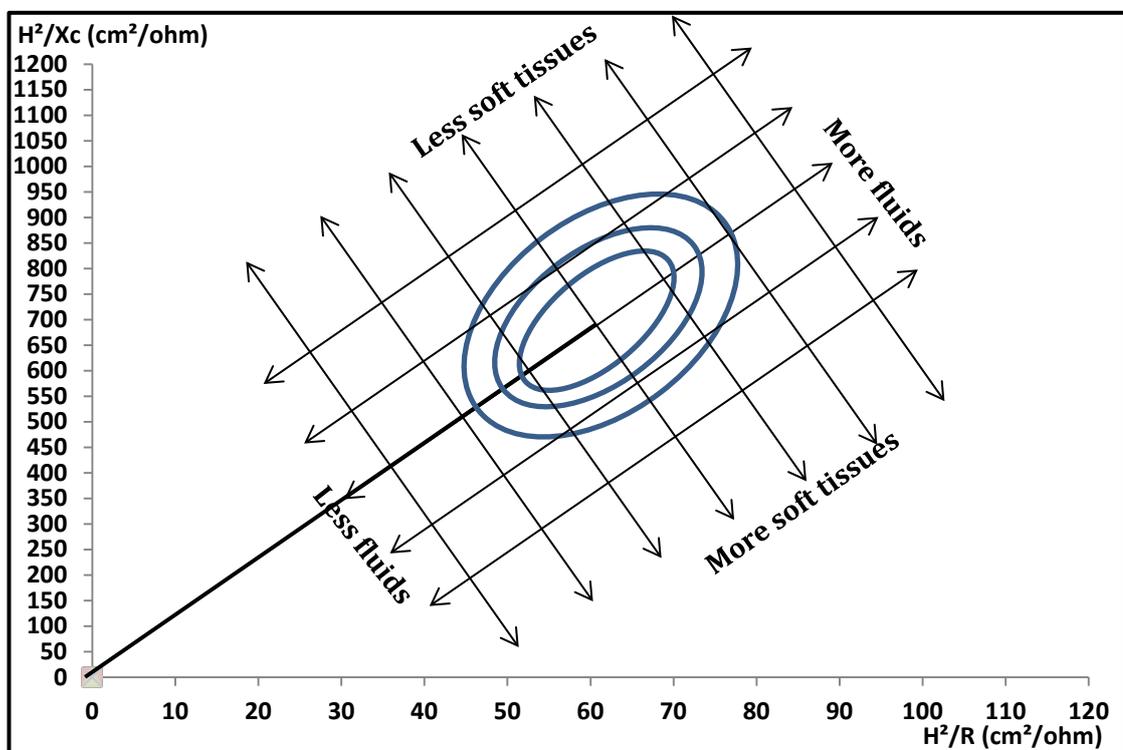


Figure 4.3 Changing BI vector plot and the associated clinical interpretation of body composition.

If the patient weight remains stable then this interpretation is relatively straight forward; for example if the vector lengthened but the weight is unchanged this implies more fluid is present, and the change in the vector angle indicates whether this is extracellular or intracellular. As clinicians were asked to keep the vector stable this would have led to a reduction in target weight if the vector widened. However if the patient's weight changed

spontaneously then the interpretation of the data was done with the help of the algorithm shown in Figure 4.4.

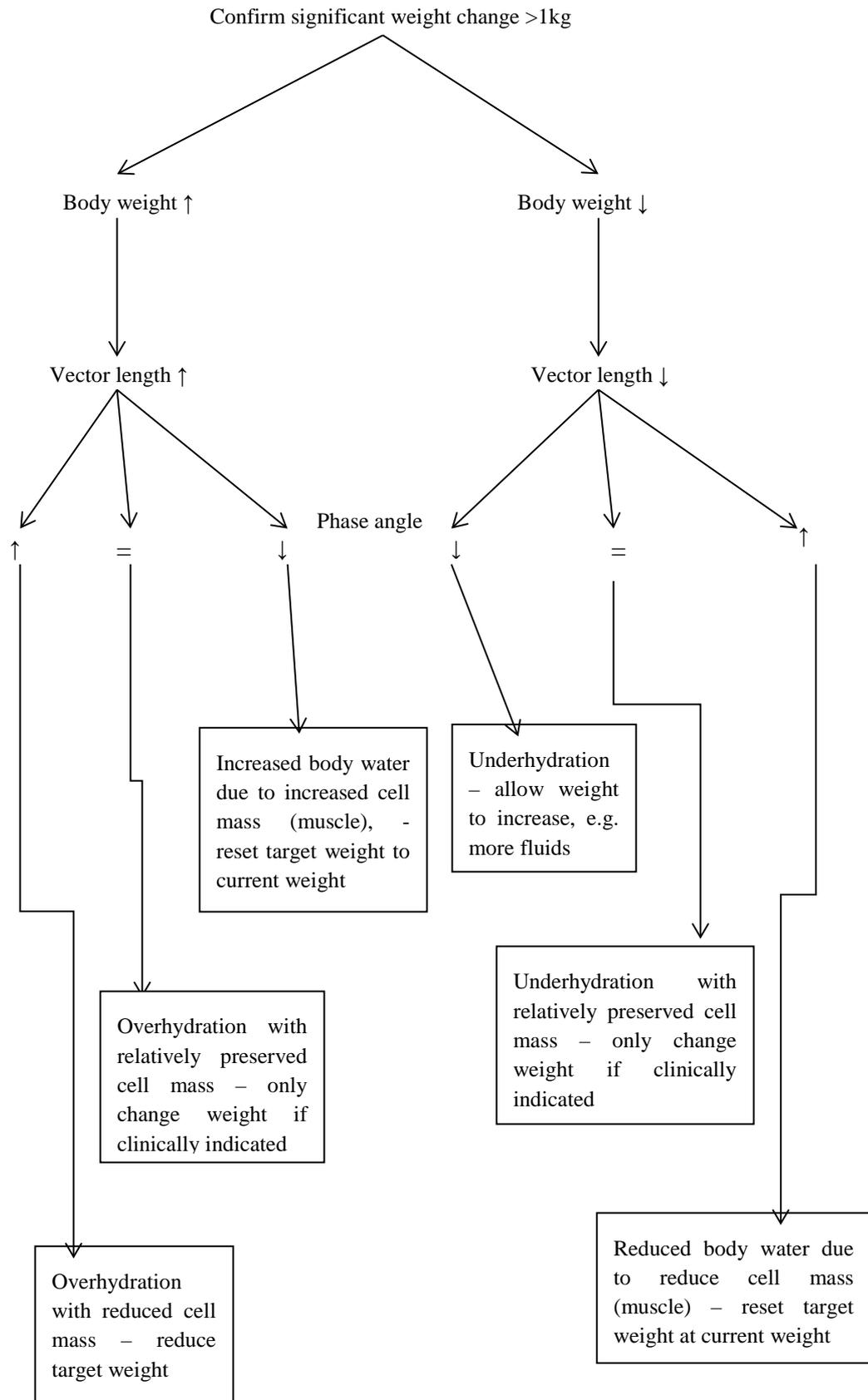


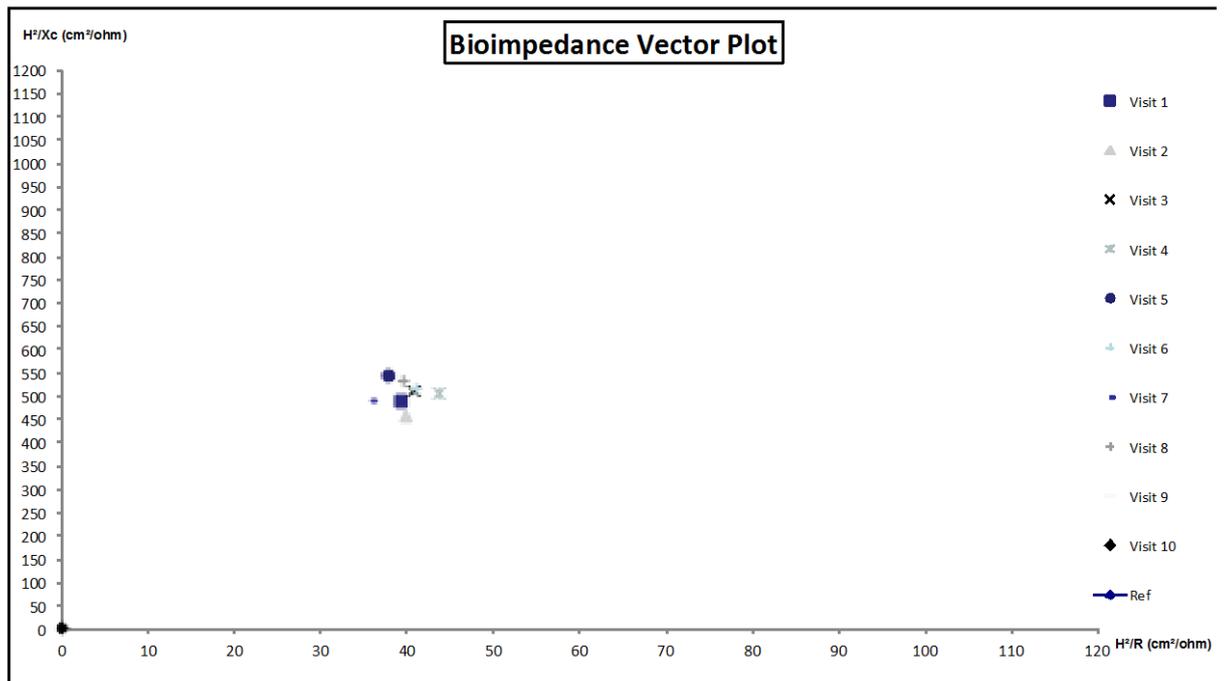
Figure 4.4 Algorithm to facilitate clinical decision making.

In this way changes (or not) in actual bodyweight and clinical examination can be interpreted alongside trends but not absolute measures of body composition. The interventions employed by clinicians were at their discretion and availability but included adjustment of dry weight (e.g. by asking the patient to reduce salt and fluid intake), altering the dialysis prescription of glucose or icodextrin, changing modality (CAPD or APD) or altering diuretic prescription (see Table 4.7 for summary of practice patterns). These were recorded along with the rationale behind the decision.

4.3.4 Examples of longitudinal vector plots and how they supported clinical decision making

Case 1

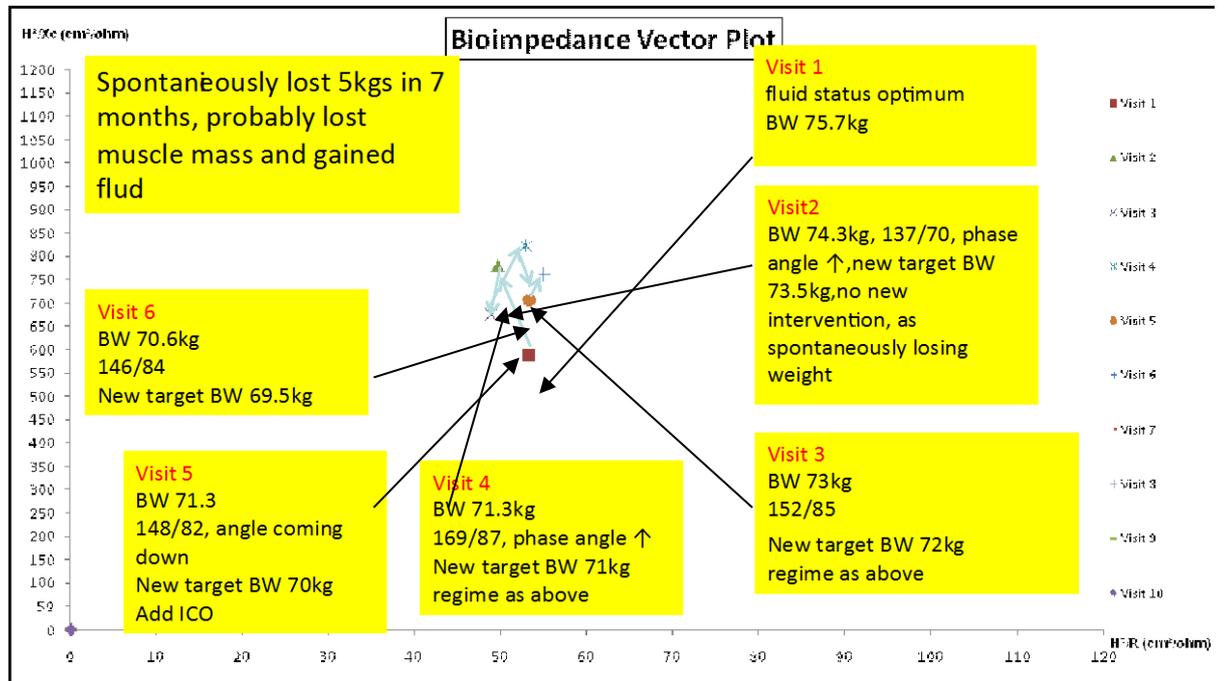
83 yr female. 6 months in study. Maintain target weight at 50kg.
Serial vectors largely unchanged.



In this case the vector and the weight remained very stable so no interventions were made.

Case 2

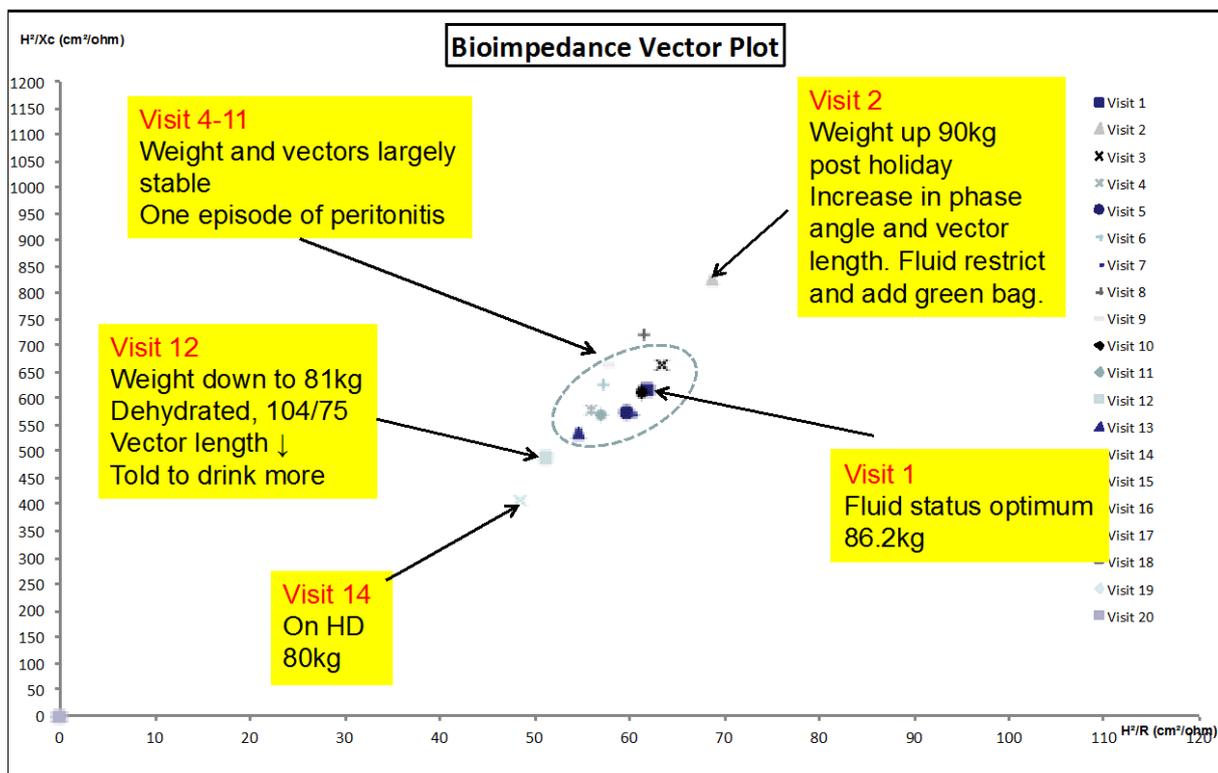
85 yr with chronic lymph oedema Comorbidity Score 1



In this example the patient spontaneously lost weight, but the vector was kept stable by re-setting the target weight in a downwards direction.

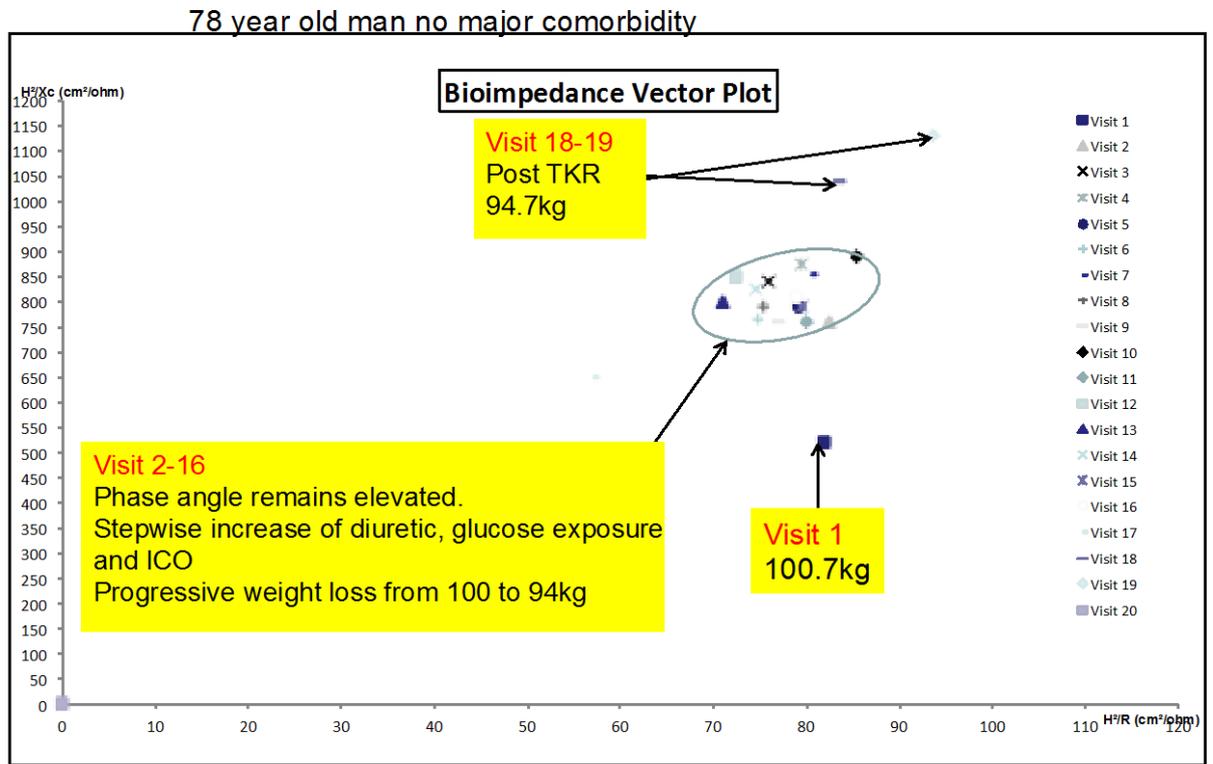
Case 3

61 yr male, 5 years on PD, anuric, transferred to HD in August due to recurrent peritonitis



In this example there was an initial increase in weight associated on the vector plot with overhydration – reported increase in fluid intake on holiday - treated by fluid restriction and stronger dialysate glucose prescription. There then followed a period of stability, but subsequently he became dehydrated. He then switched temporarily to haemodialysis for a period – assessment 14 – associated with a reduction in the dry weight, and later returned to PD (data not shown) and stable vector.

Case 4



At visit 2 considered to have become overhydrated despite no change in weight. Despite progressive reduction in the target weight and interventions to get better fluid control – which stabilised the vector – there was further deterioration in his fluid status associated with a total knee replacement (TKR), during which is albumin dropped significantly postoperatively as part of an inflammatory response to infection.

4.4 Materials and methodology

4.4.1 Bioimpedance Measurements

All units were issued with BI 101 ASE (Anniversary Sport Edition, Akern, Italy) body composition analyzers. These devices measure resistance and reactance at a single (50

MHz) frequency utilising the RJL Quantum technology previously validated in dialysis patients (Chertow, Lowrie et al. 1995, Mushnick, Fein et al. 2003, Jankowska, Debska-Slizien et al. 2006). BI measurements were taken using a standardized protocol, with documentation of the placement of electrodes, without draining the abdomen of dialysate and after the patient had been lying recumbent for at least 5 minutes. Using this approach we have previously documented within patient on the same day CV of <1% and inter-class correlations to determine intra-observer error of >0.96.

4.4.2 Clinical measurements

Comorbidity was characterized using the externally validated Stoke scoring system (Davies, Phillips et al. 2002). Blood pressure was measured in clinic attendance using standard equipment. Residual renal function was determined from 24-hour urine collections and calculated as the mean urea and creatinine clearance normalized to body surface area of 1.73m². Dialysis dose was determined from 24-hour collections and expressed as weekly Kt/V_{urea}. Membrane function was determined using the peritoneal equilibration test and expressed as the four hour dialysate: plasma creatinine ratio (solute transport rate) and net ultrafiltration corrected for overfill (ultrafiltration capacity). Glucose utilization was calculated from the product of the volume and the concentration and expressed as grams used per day. Plasma albumin was determined using the bromocresol purple (BCP) colorimetric method in the UK and bromocresol green (BCG) method in Shanghai. The systematic difference between these methods is Alb_{BCG}=5.5+Alb_{BCP} (Clase, St Pierre et al. 2001), but data here is expressed after correction to the BCG method to enable comparison.

4.5 Statistical analysis and power calculations

The trial was powered so as to be able to detect a clinically meaningful longitudinal change in body composition from baseline, specifically ECW volume, with an *a priori* assumption that spontaneous changes would occur in the control group that would be prevented in the active group. Based on pilot data obtained from longitudinal studies of body composition in PD patients over 12 months in which the SD of the difference in ECW was 1.22 L, then to detect a 1 L difference in ECW, assuming a 5% type 1 error with 80% power would require a 25 patients per group with 1:1 randomization; to detect a 0.8 L change would require 38 in each arm (WinSSD version 1.6.1). The plan was to recruit sufficient numbers to detect a 1 kg difference, but allow over-recruitment within the predefined window. For non-anuric and anuric patients the anticipated drop-out was 25% and 35% respectively necessitating ~130-150 patients enrolling per country.

The predefined primary analysis was planned as follows: after study completion and data lock (August 2012) the *R* and *X_c* data were converted to estimates of ECW, TBW, (and ECW/TBW) using an algorithm based on the software provided with the BI device and the change at 12 months from baseline determined by paired t-tests, to include changes in target and actual weight and secondary outcomes. To assess drop-out between group differences in patient and study survival were determined from Kaplan Meier survival curves and the log rank test. Mean values and frequencies of parameters including fluid assessments and interventions were compared using ANOVA or χ^2 tests as appropriate. A secondary multi-level regression analysis with BI derived fluid volumes as the dependent variables was undertaken to include all observations (level 1), individuals (level 2) and centres (level 3) and adjustment for the baseline covariates associated with BI volumes on

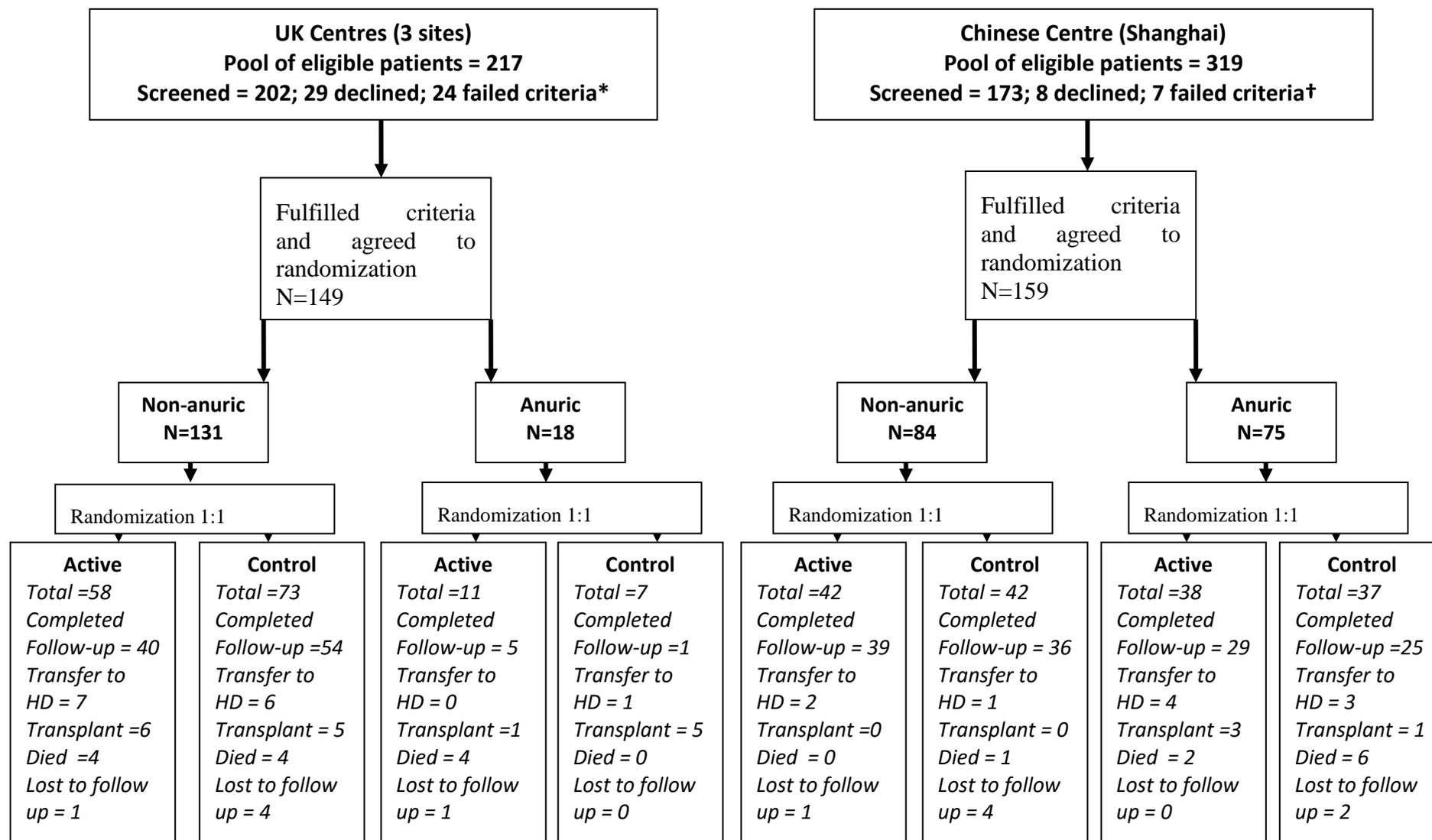
exploration of the dataset: age, gender and comorbidity. Models were fitted separately for active and control limbs in anuric and non anuric groups with random intercepts at individual and centre. All statistical analyses were undertaken using SPSS software version 20, (IBM, US), except for the multilevel analysis for which we used MLwin (Version 2.22, Centre for multilevel modelling University of Bristol, UK).

4.6 Results

4.6.1 Patient characteristics.

Recruitment, randomisation and dropout to and from the four study groups are summarised in the consort diagram (Figure 4.5). With the exception of the UK-anuric group, recruitment was sufficient to test our primary outcome with 80% power to detect a 1 kg change (in ECW) in the Shanghai (non-anuric and anuric) patients and 0.8 kg change in the UK non-anuric (UK-non-anuric) patients. Failure to achieve power in the UK-anuric group was due to a combination of lack of recruitment indicative of the low proportion of anuric patients in the 3 UK centres and a high dropout (66%). There was a non-significant increase in deaths in this patient group randomised to the BI intervention; careful analysis of these 4 deaths (cancer, sepsis) and adverse outcomes did not indicate any common factor or plausible relationship to the intervention but this group was excluded from further analysis apart from the multivariate models. Dropout in the remaining groups was well balanced over the course of the study as shown by Kaplan-Meier plots and log-rank tests (Figure 4.6).

Figure 4.5 Consort Diagram (failed criteria *unlikely to remain on PD for 6 months due to planned transplant or modality transfer, † unable to achieve clinical euvolaemia during run in).



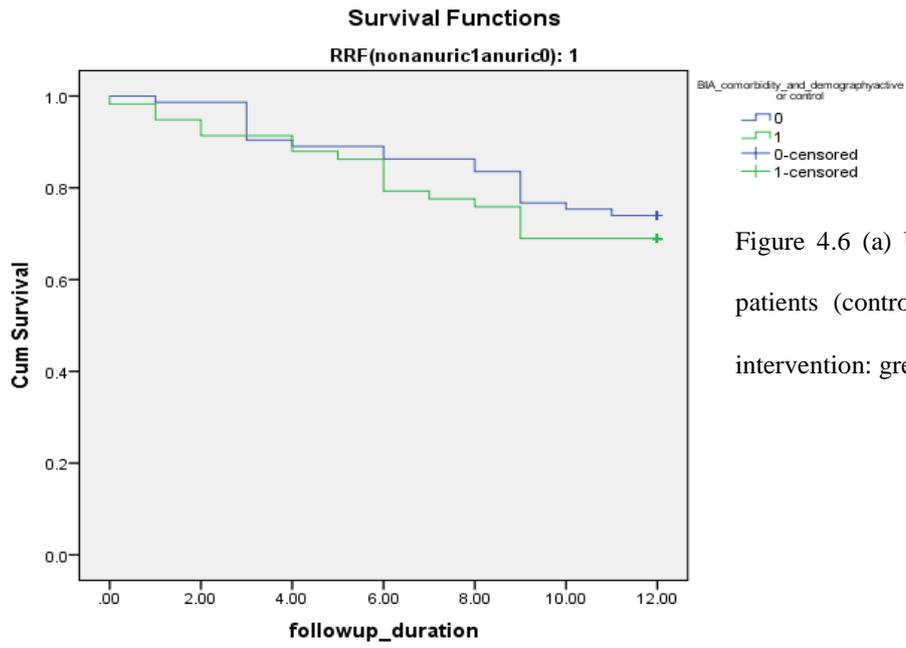


Figure 4.6 (a) UK-Non-Anuric patients (controls: blue; BIA intervention: green).

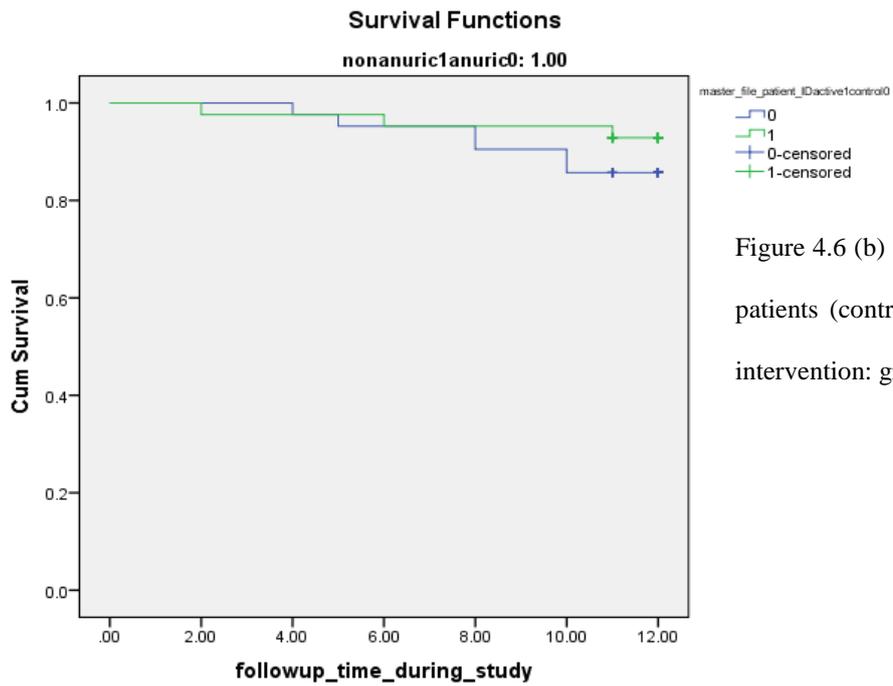


Figure 4.6 (b) SH-Non-Anuric patients (controls: blue; BIA intervention: green).

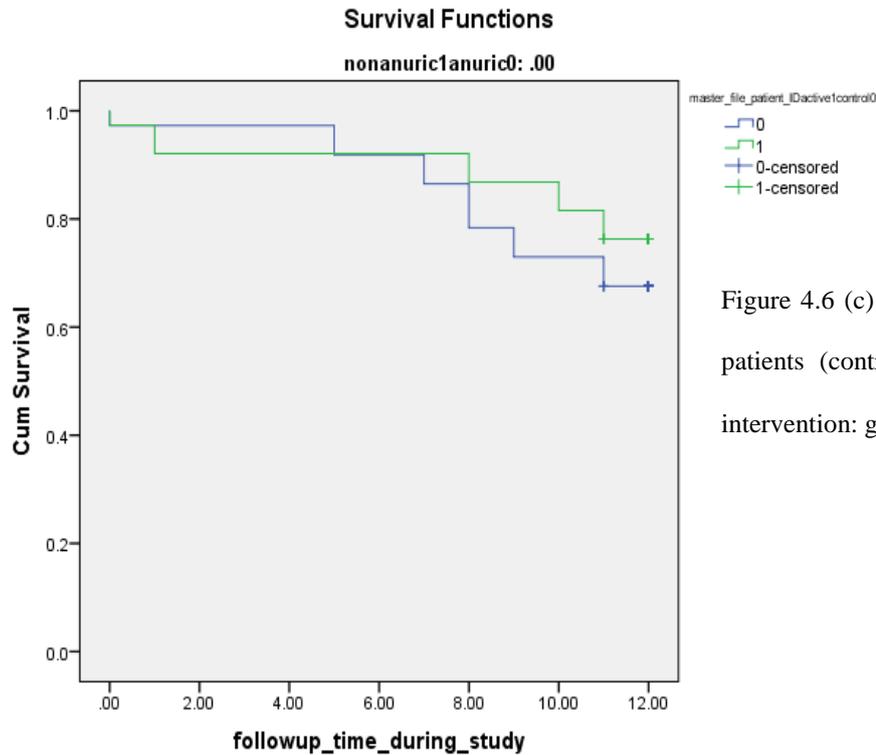


Figure 4.6 (c) Shanghai-Anuric patients (controls: blue; BIA intervention: green).

Figure 4.6 Kaplan Meier survival plots for patient drop out by group.

There were no significant differences between patients randomised to the BI-intervention or control arm in any of the groups in terms of their baseline demography, dialysis prescription, residual kidney function, peritoneal membrane function, blood pressure or body composition (see Table 4.1). Shanghai patients tended to be younger (mean age 54.0 v. 58.6 years), have less comorbidity (20% v. 60% with at least one other diagnosis) and weigh significantly less than UK patients (58.9 v. 76.8 kg) which was reflected in a lower dialysis prescription volume. The average blood pressure and peritoneal solute transport rates (PSTR) were lower in the SH patients. Comparing the non-anuric patients, normalised residual renal clearances were higher in UK patients, as was the absolute residual urine volume unless this was corrected for body weight when the difference was non-significant (16.7 v. 17.4 ml/kg). As expected, anuric patients had been on peritoneal

dialysis for longer, Shanghai-anuric 58 (26-90) v. Shanghai-non-anuric 19 (7-41) and UK-anuric 57 (36-72) v. UK-non-anuric 22 (7-33) months.

Table 4.1 Baseline characteristics of patient groups according to randomisation

	UK-NON-ANURIC		UK-ANURIC		SH-NON-ANURIC		SH-ANURIC	
	INTERVENTION	CONTROL	INTERVENTION	CONTROL	INTERVENTION	CONTROL	INTERVENTION	CONTROL
N	58	73	11	7	42	42	38	37
PD duration (months, range)	18 (7-32)	22(8-34)	67(34-81)	45(36-60)	17(7-37)	24(11-41)	52(22-86)	65(31-105)
age (year)	58.3±15.3	56.9±14.7	64±14.7	56.4±11	56.6±11.2	52.5±14.4	51.1±13.1	55.5±13.5
D/P creatinine	0.70±0.12	0.68±0.12	0.69±0.1	0.79±0.07	0.60±0.09	0.58±0.11	0.61±0.09	0.65±0.11
albumin (g/L)	33.41±4.47	33.48±3.85	33.4±1.9	34.3±5.2	32.57±2.90	32.54±3.61	32.8±4.3	33.4±2.9
Gender F/M	20/38	29/44	7/4	4/3	26/16	31/11	19/19	14/23
Comorbidity (low/med/high)	25/29/4	28/39/6	5/6/0	2/5/0	34/7/1	35/7/0	31/7/0	27/10/0

	UK-NON-ANURIC		UK-ANURIC		SH-NON-ANURIC		SH-ANURIC	
	INTERVENTION	CONTROL	INTERVENTION	CONTROL	INTERVENTION	CONTROL	INTERVENTION	CONTROL
ECW (L)	18.3±4.3	19.2±4.0	16.4±4.0	14.5±3.0	16.7±3.1	15.9±2.6	16.3±3.2	17.4±3.5
TBW (L)	41.5±8.2	42.3±8.5	37.4±5.6	36.1±8.6	35.0±5.2	33.9±4.8	34.6±6.6	35.8±6.9
ECW/TBW	0.44±0.07	0.46±0.06	0.45±0.1	0.42±0.1	0.48±0.06	0.47±0.05	0.47±0.06	0.49±0.06
H²/R (cm²/Ω)	58.6±13.6	59.6±13.2	51.9±9	47.6±12	49.4±11.8	48.8±8.1	49.5±12.3	51.9±12.4
H²/Xc (cm²/Ω)	538.9±172	570.1±162	487.7±164	384.2±102	526.3±132	497.5±129	502.6±135	560.9±159
Target weight (kg)	76.2±15.7	78.4±18.5	72.4±12.2	72.4±20.6	59.9±8.2	58.4±8.9	58.0±9.5	59.0±11.0
Systolic BP (mmHg)	136.8±20.1	143.4±22.5	131.8±28	136.4±16.6	130.2±14.8	130.5±17.7	128.3±20.4	127.2±23.0
Diastolic BP (mmHg)	79.7±11.4	81.3±12.3	75.0±10.3	73.9±12.3	82.7±11.2	83.5±9.5	79.9±12.1	79.5±10.2

	UK-NONANURIC		UK-ANURIC		SH-NONANURIC		SH-ANURIC	
	INTERVENTION	CONTROL	INTERVENTION	CONTROL	INTERVENTION	CONTROL	INTERVENTION	CONTROL
N	58	73	11	7	42	42	38	37
Diuretics (%)	54.3	54.8	9.1	0	29.3	28.6	0	2.6
Beta-blockers (%)	25.7	27.4	18.2	0	34.1	26.2	18.9	26.3
ACE-inhibitors (%)	24.3	21	0	28.6	9.8	21.4	24.3	18.4
ARBs (%)	18.6	12.9	18.2	14.3	46.3	50	24.3	48.4
Calcium-channel blockers (%)	22.9	24.2	6.4	14.3	63.4	71.4	59.6	71.1
Alpha-blockers (%)	14.3	21	18.2	0	2.4	4.8	0	2.6

ECW = Extracellular water, TBW = Total Body Water, H^2/R = Height² /Resistance, H^2/Xc = Height² /Reactance; ACE = Angiotensin converting enzymes; ARB = Angiotensin Receptor Blocker

4.6.2 Primary outcome; longitudinal change in ECW and body composition

In both the UK- and Shanghai-non-anuric controls and the Shanghai BI intervention group there were no significant changes in body composition over the 12-month study period (see diagram a and b in Figure 4.7 and Table 4.2). In the UK-non-anuric BI intervention group there was a reduction in TBW (-0.9 kg, 95%CI: 0 to - 1.74) that was associated with a reduced target weight set by clinicians (-1.7 kg, 95%CI: -0.39 to -2.96) and actual weight (-1.3 kg 95%CI: -0.09 to -2.69). Despite this there was no change in the ECW (+0.3 kg, 95%CI: -0.69 to 1.24) or the ECW/TBW (0.01 95%CI: -0.04 to 0.01 Figure 4.7-a). In the Shanghai-anuric patients a significant deterioration in body composition occurred in the control patients due to a fall in TBW (-1.76kg, 95%CI: -2.70 to -0.82), increase in ECW (+0.59kg, 95%CI: -0.67 to 1.86) and thus worsening of the ECW/TBW ratio (0.04, 95%CI: 0.01 to 0.06), whereas body composition remained stable in the BI intervention group (Figure 4.7-c). This was associated with stability of the BI vector plot used to aid clinical decision making in the intervention group whereas in the controls there was a reduction in $\text{Height}^2/\text{Resistance}$ in keeping with reduced muscle mass and worsening in the phase angle (-0.58° , 95% CI: 0.08° to 1.07°), indicating a relative excess in tissue hydration (Figure 4.2). On multilevel analysis combining the data from both countries and all study visits these differences remained significant and independent of baseline determinants of fluid volumes including gender, age and grade of comorbidity (Table 4.3). By visit 5 (12 months) the ECW/TBW ratio worsened significantly in both the anuric control and intervention group.

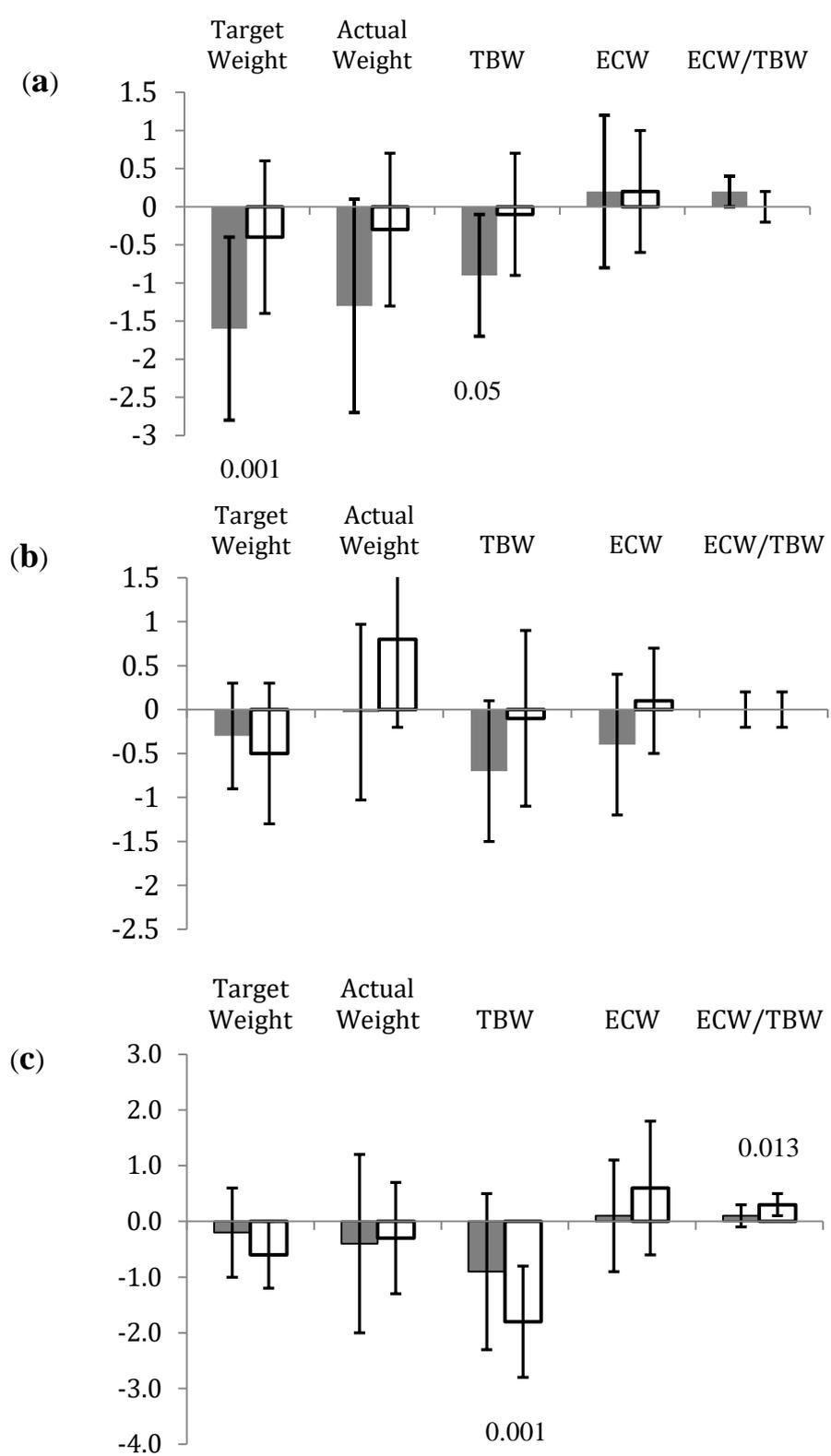


Figure 4.7 Net changes in body composition (weight in Kg, fluid volumes in L) at 12 months for (a) UK-NonAnuric, (b) Shanghai-NonAnuric and (c) Shanghai-Anuric patients (Active BIA intervention group: solid bars, Controls: open bars, \pm 95% CI).

Table 4.2 Longitudinal changes on body composition

	UK-Non Anuric						Shanghai – Non Anuric						Shanghai – Anuric					
	Control			BI Intervention			Control			BI Intervention			Control			BI Intervention		
	Base line	12 month	P	Baseline	12 month	P	Baseline	12 month	P	Base line	12 month	P	Base line	12 month	P	Baseline	12 month	P
ECW (L)	19.5 ±4.2	19.7 ±4.6	0.70	18.2 ±4.3	18.4 ±4.0	0.57	16.0 ±2.4	16.1 ±2.6	0.84	17.2 ±2.5	16.7 ±3.2	0.25	17.7 ±3.2	18.3 ±4.6	.342	16.3 ±3.2	16.2 ±2.6	.787
TBW (L)	42.9 ±8.6	42.8 ±8.8	0.92	41.3 ±8.4	40.4 ±7.9	0.05	34.3 ±6.6	34.2 ±6.9	0.82	35.4 ±5.2	34.7 ±5.8	0.10	37.2 ±6.0	35.5 ±5.9	.001	35.0 ±6.5	34.0 ±5.5	.179
ECW/ TBW	0.46 ±0.06	0.46 ±0.07	0.56	0.44 ±0.08	0.46 ±0.06	0.17	0.47 ±0.06	0.47 ±0.06	0.75	0.48 ±0.08	0.48 ±0.07	0.89	0.48 ±0.06	0.51 ±0.09	.013	0.47 ±0.06	0.48 ±0.08	.221
H²/R (cm²/Ω)	59.8 ±14.0	59.9 ±14.4	0.93	58.3 ±14.5	56.9 ±13.8	0.13	49.5 ±17.4	48.9 ±10	0.61	48.5 ±10.4	49.3 ±9.8	0.08	54.7 ±10.5	50.4 ±9.8	.001	50.5 ±12.3	48.5 ±10.4	.168

	UK-Non Anuric						Shanghai – Non Anuric						Shanghai – Anuric					
	Control			BI Intervention			Control			BI Intervention			Control			BI Intervention		
	Baseline	12 month	P	Base line	12 month	P	Baseline	12 month	P	Base line	12 month	P	Base line	12 month	P	Baseline	12 month	P
H²/X (cm²/Ω)	576 ±171	595 ±221	0.39	539 ±175	543 ±176	0.88	499 ±127	498 ±154	0.98	538 ±146	529 ±183	0.72	567 ±147	621 ±253	.209	505 ±139	511 ±145	.815
Phase angle (degrees)	6.14 ±1.2	6.13 ±1.55	0.91	6.59 ±2.08	6.25 ±1.35	0.25	6.14 ±1.20	6.13 ±1.55	0.82	5.50 ±0.82	5.66 ±1.49	0.42	5.72 ±1.30	5.15 ±1.72	0.03	5.90 ±1.36	5.75 ±1.68	0.50
Target weight (kg)	78.7 ±19	78.3 ±19	0.44	75.2 ±15	73.6 ±15	0.01	59.2 ±9.0	58.7 ±9.1	0.20	60.7 ±8.6	60.3 ±8.6	0.30	61.4 ±10.2	60.8 ±10.5	.099	58.3 ±9.8	58.1 ±8.9	.588
Clinical weight (kg)	79.3 ±18	79.0 ±19	0.72	75.8 ±16	74.5 ±15	0.07	59.2 ±9.0	60 ±9.7	0.13	60.7 ±8.6	60.6 ±8.8	0.94	61.2 ±10.4	60.9 ±10.9	.586	58.3 ±9.8	57.9 ±8.4	.622

ECW = Extracellular water, TBW = Total Body Water, H²/R = Height²/Resistance, H²/Xc = Height²/Reactance Phase angle is calculated as the arc tangent ((H²/R)/(H²/Xc)) expressed in degrees.

Table 4.3 Multilevel analysis of determinants of BI derived fluid volumes (TBW and ECW/TBW) incorporating all visits (level 1), patients (level 2) and centres (level 3).

	Control (All)	BI Intervention (All)	Controls (Non-Anuric)	BI intervention (Non-Anuric)	Controls (Anuric)	BI intervention (Anuric)
Number of patients	158	149	115	100	44	49
Number of visits	666	637	491	435	175	202
Mean TBW (L, 95%CI)						
Baseline constant	34.7 (31.6, 37.8)	34.1 (31.1- 37.0)	33.7 (31.0, 36.5)	33.6 (30.4, 36.7)	31.8 (29.3, 34.3)	33.1 (29.6, 36.7)
Gender (male)	10.1* (8.4, 11.9)	8.8 * (7.2 -10.4)	11.7* (9.6, 13.9)	9.2* (7.1, 11.3)	8.6 * (5.8, 11.4)	8.8* (6.4, 11.3)
Age (year)	0.06 (0.0, 0.12)	-0.01 (-0.07- 0.05)	0.00 (-0.07, 0.07)	-0.01 (-0.09, 0.06)	0.21* (0.09, 0.32)	-0.03 (-0.12, 0.06)
Comorbidity Grade 1 †	-0.84 (-2.81, 1.13)	0.73 (-1.13, 2.60)	0.38 (-1.91, 2.67)	1.86 (-0.42, 4.14)	-2.46 (-5.55, 0.63)	-1.70 (-4.59, 1.18)
Comorbidity Grade 2 †	-1.96 (-6.27, 2.4)	0.95 (-3.63, 5.53)	-1.53 (-5.86, 2.80)	1.43 (-3.33, 6.20)	None in this category	
Visit 2 v. baseline§	0.40 (-0.07, 0.86)	-0.12 (-0.55, 0.30)	0.44 (-0.08, 0.96)	-0.44 (-0.91, 0.03)	0.23 (-0.72, 1.19)	0.57 (-0.30, 1.43)
Visit 3 v. baseline§	0.11 (-0.36 - 0.59)	-0.41 (-0.83, 0.0)	0.12 (-0.41, 0.65)	-0.57† (-1.03, -0.10)	0.06 (-0.91, 1.03)	-0.08 (-0.92, 0.76)
Visit 4 v. baseline§	-0.45 (-0.95- 0.05)	-0.51† (-0.9, -0.06)	-0.13 (-0.68, 0.42)	-0.52† (-1.01, -0.02)	-1.49* (-2.58, -0.41)	-0.46 (-1.35, 0.42)
Visit 5 v. baseline§	-0.45 (-0.96 - 0.06)	-0.79† (-1.24, -0.4)	-0.15 (-0.71, 0.41)	-0.81* (-1.30, -0.31)	-1.52* (-2.67, -0.37)	-0.79 (-1.70, 0.12)
ECW/TBW (ratio, expressed as percentage, 95%CI)						
Baseline constant	46.3 (43.8, 48.7)	46.4 (43.9, 48.8)	46.9 (45.1, 48.7)	46.3 (43.8, 48.9)	47.6 (42.1, 53.1)	46.8 (43.0, 50.5)
Gender (male)	-2.78 * (-4.37, -1.18)	-3.32* (-4.97, -1.67)	-3.53* (-5.36, -1.69)	-2.58* (-4.54, -0.62)	-3.68† (-7.00,-0.36)	-4.26* (-7.37, -1.14)
Age (year)	0.07† (0.01, 0.12)	0.14* (0.08, 0.20)	0.06† (0.00, 0.12)	0.12* (0.05, 0.19)	0.05 (-0.08, 0.18)	0.17* (0.05, 0.28)
Comorbidity Grade 1 †	1.54 (-0.28, 3.36)	2.30† (0.43, 4.16)	1.15 (-0.79, 3.08)	1.31 (-0.81, 3.42)	1.21 (-2.62, 5.05)	3.97† (0.33, 7.62)
Comorbidity Grade 2 †	8.81* (4.83, 12.78)	1.61 (-2.96, 6.18)	8.72* (4.97, 12.46)	1.71 (-2.69, 6.11)	None in this category	
Visit 2 v. baseline§	0.08 (-0.89, 1.04)	0.57 (-0.43, 1.57)	-0.29 (-1.37, 0.79)	0.32 (-0.93, 1.57)	1.05 (-0.97, 3.06)	1.11 (-0.51, 2.72)
Visit 3 v. baseline§	0.52 (-0.46, 1.51)	-0.09 (-1.07, 0.89)	0.44 (-0.67, 1.55)	-0.26 (-1.49, 0.97)	0.74 (-1.29, 2.78)	0.35 (-1.22, 1.91)
Visit 4 v. baseline§	-0.49 (-1.53, 0.55)	0.45 (-0.58, 1.48)	-0.39 (-1.54, 0.76)	0.37 (-0.94, 1.67)	-0.83 (-3.10, 1.45)	0.71 (-0.94, 2.36)
Visit 5 v. baseline§	0.96 (-0.10, 2.03)	0.85 (-0.19, 1.90)	0.31 (-0.85, 1.47)	0.50 (-0.81, 1.81)	3.25* (0.85, 5.66)	1.79† (0.09, 3.48)

6 models are presented; in each case the BI fluid volume (TBW = Total Body Water, ECW = Extracellular water) is adjusted for baseline age, gender and comorbidity († compared to Grade 0 = no comorbid conditions, Grade 1 = 1-2, Grade 2 = 3 or more comorbidities); TBW at baseline is greater by 8.5 – 10 litres in men and falls over visits (spontaneously in anurics, due to target weight reduction in non-anurics) whereas the ECW/TBW at baseline is elevated by age, female gender and comorbidity and increases over visits in anuric patients, § = Change from baseline visit; †P<0.05, *P<0.01.

4.6.3 Secondary clinical outcomes – blood pressure, residual kidney function, membrane function and dialysis dose

There were no significant longitudinal changes in blood pressure in any of intervention or control groups (Table 4.4). In all the non-anuric patient groups over 12 months there was a significant fall in the residual Kt/V_{urea} associated with reductions in urine volume with the exception of the UK intervention group in whom residual urine volume was maintained. Although relative preservation of urine volume in the context of loss in solute clearance is in keeping with increased diuretic use it was not possible to demonstrate this (Table 4.4). The reduction in residual function in the non-anuric groups was associated with increases in the prescribed dialysis dose (volume) in all patient groups. There were no changes in plasma albumin, ultrafiltration capacity or PSTR in any of the groups with the exception of a significant increase in PSTR in the Shanghai-anuric cohort, seen in both study arms, slightly greater in the intervention arm.

Table 4.4 Longitudinal changes in secondary outcomes and dialysis prescription. NC=not collected.

	UK-Non Anuric						Shanghai – Non Anuric						Shanghai – Anuric					
	Control			BI Intervention			Control			BI Intervention			Control			BI Intervention		
	Baseline	12 month	P	Baseline	12 mon	P	Baseline	12 month	P	Baseline	12 month	P	Baseline	12 month	P	Baseline	12 month	P
Systolic BP (mmHg)	144 ±25	140 ±20	.209	137 ±20	136 ±20	.845	133 ±16.2	135 ±18.4	.375	131 ±15	130 ±21	.637	128 ±24	123 ±27	.638	130 ±22	128±26	.776
Diastolic BP (mmHg)	81.2±13	79.1±10	.235	79.7±11	79.2±11	.815	85.5±8	86.1±13	.991	83.3±11	81.9±11	.410	79.7±9	76.2±16.2	.246	79.8±13.1	81.6±14.9	.613
Input Volume (L)	8.8 ±2.5	9.4 ±3	.032	8.7 ±2.7	9.0 ±3	.079	6.1 ±1.8	6.5 ±1.8	.025	6.6 ±1.5	6.9 ±1.4	.025	8.3 ±1.3	8.6 ±1.4	.083	7.8 ±1.2	8.0 ±1.2	.157
Total Glucose (g)	121.7±5	126.7±6	.395	119.1±51	126.8±57	.067	104.2±35	120.0±39	.001	116.0±31	130.0±36	.003	181.2±41	186.7±51	.398	167.5±39	176.3±46	.037
Average [Glucose] (g/L)	1.36±0.28	1.3±0.4	.257	1.31±0.37	1.34±0.39	.417	1.71±0.32	1.86±0.34	.009	1.75±0.27	1.87±0.34	.006	2.16±0.31	2.15±0.39	.888	2.13±0.33	2.19±0.39	.342
daily UF(UF)	326 ±629	446 ±435	.113	339 ±539	439 ±418	.203	160 ±437	352 ±487	.003	166 ±514	316 ±568	.012	876.4 ±296	792 ±402	.384	833 ±327	831 ±363	.819
Urine Volume(ml)	1,298 ±746	1,007 ±599	.003	1,165 ±661	1,170 ±896	.957	1,084 ±621	746 ±559	.000	1,005 ±571	723 ±654	.000	NC	NC		NC	NC	
Renal Ccr (ml)	94.6±63	78.1±57	.020	93.1±66.1	72.3±62.6	.001	40.1±35.3	33.9±38.7	.012	41.7±33.1	27.2±26.5	.000	NC	NC		NC	NC	
Renal KtV	1.3±0.8	1.1±0.7	.023	1.2±0.8	1.0±0.8	.006	0.8±0.6	0.6±0.6	.007	0.7±0.5	0.5±0.5	.000	NC	NC		NC	NC	
Albumin (g/L)	33.5±4.0	32.9±3.7	.301	33.2±4.7	33.2±5.0	1.00	38.0±3.4	38.2±4.6	.466	37.8±2.8	38.4±3.3	.307	38.9±3.3	38.2±4.3	.548	39.2±3.0	38.0±3.5	.130
UF capacity	274 ±216	244 ±220	.572	226 ±266	279 ±294	.357	253 ±111	291 ±138	.147	221 ±174	266 ±130	.299	274 ±139	252 ±153	.701	241 ±196	267 ±129	.247
Solute Transport	0.66±0.11	0.68±0.10	.156	0.70±0.14	0.73±0.11	.175	0.58±0.11	0.60±0.12	.231	0.60±0.10	0.62±0.10	.255	0.64±0.11	0.67±0.11	.032	0.62±0.11	0.67±0.11	.001

4.6.4 Analysis of interventions related to fluid management

A key aspect of the study design was to capture decisions at the point of fluid management assessments so as to better understand how clinicians were utilising the additional information from BI. Of a potential 1,274 visits and 1,394 assessments (additional assessments were allowed in the active BI group) of fluid status, data was captured and recorded for analysis at 1,106 visits (89.6%), 568 for Shanghai with >95% data validity, 538 for the UK with 85% validity, proportions not significantly different by randomisation group. Frequency of intervention type were highly variable by patient, usually multiple and often repeated (see Table 4.5), but not demonstrably different according to randomisation, with the exception of significantly higher glucose prescription in the Shanghai-anuric BI group (Table 4.4). By taking a change in target weight of ≥ 1 kg in either direction as a cut-off value for a clinically significant decision, it can be seen (Table 4.6) that the weight was more likely to be reduced in the intervention compared to control groups in all three sub-studies with the primary reason given as ‘to improve control of high blood pressure’, whereas hydration status on clinical examination was less likely to be a factor (although reduction of weight in the Shanghai-anuric BI group was borderline significant). Overall this effect on decision making was most marked in the UK-non-anuric BI patients, so explaining the overall greater likelihood of weight reduction in this intervention group as already discussed (Figure 4.7-a).

Table 4.5 Clinical interventions by different centres.

Intervention	UK Centres	Shanghai	Total
Reduce Fluid Intake	14.8%	29.9%	22.5%
Increase Fluid Intake	6.8%	1.8%	4.4%
Commence/increase diuretics*	2.4%	2.2%	2.3%
Reduce/stop diuretics	1.0%	-	0.05%
Increase Dialysate Glucose prescription	14.5%	7.9%	11.3%
Decrease Dialysate Glucose prescription	1.0%	0.07%	0.085%
Commence icodextrin	0.04%	N/A	
Decrease icodextrin	0.03%	N/A	

* Denominator adjusted for number of interventions in non-anuric patients.

N/A = not applicable as unavailable in China

Table 4.6 Analysis of interventions according to changes in target dry weight defining $\geq \pm 1$ kg as being clinically significant. \downarrow : target weight decreased by ≥ 1 kg; =: change in target weight < 1 kg; \uparrow : target weight increased by ≥ 1 kg P=values are for the χ^2 test. ND=Not done

Clinical decision	Randomisation	UK-NA				S-NA				S-A				All Groups			
		\downarrow	=	\uparrow	P value	\downarrow	=	\uparrow	P value	\downarrow	=	\uparrow	P value	\downarrow	=	\uparrow	P value
Reason given for changing weight to achieve lower blood pressure	BI intervention	10	30	1		6	27	1		6	29	3		22	86	5	
	Controls	13	31	12	0.004	11	36	4	0.03	2	26	1	0.002	26	93	17	0.008
Reason given for changing weight to achieve better fluid status based on clinical examination	BI intervention	6	20	1		9	33	1		8	21	2		23	74	4	
	Controls	7	23	4	0.24	8	37	0	ND	4	28	2	0.056	19	88	6	0.15
Overall decision to change weight in either direction regardless of reason	BI intervention	38	159	19		27	135	13		24	110	13		89	404	45	
	Controls	31	177	36	0.003	25	142	16	0.58	19	109	13	0.51	75	428	65	0.006

Table 4.7 Summary for practice patterns used to manage fluid status by the participating centres.

Centre	Dietetic support and advice	Use of diuretics	Use of ACE/ARBs	Use of icodextrin
UK: Stoke-on-Trent	Dietician available in clinic. Advice on fluid restriction tailored to the individual aiming for salt intake of 5-7.5g depending on nutritional state	Continued on commencing PD. Usually Furosemide 240mg. Dose maximised before using >1.5% dialysate glucose. Stopped when anuric.	Used in all patients unless intolerant; antihypertensive drugs of choice	Used in all patients requiring a long dwell. Only replaced by glucose if clinically volume depleted.
UK: Leeds	Dietician available at clinic with periodic review of all patients. Salt restricted to <6g/24 hours and fluid intake allowance individualised to patient	Continued on commencing PD, and start/increase dose used before more hypertonic exchanges except where there is negative UF. Stopped when anuric.	First choice of antihypertensive agent where one is required.	Used in majority of patients having a long dwell.
UK: Sheffield	Dietician available in clinic with period review of all patients. Aim for 6g salt intake per day and no added salt.	Continued on commencing PD. Furosemide dose titrated up to 240mg daily. Dose maximised before using >1.5% dialysate glucose. Stopped when anuric.	Used in all patients unless intolerant; antihypertensive drugs of choice	Used in majority of patients having a long dwell.
China: Shanghai	Dietician not available in clinic. Nursing staff and physician give advice on fluid restriction and salt intake. Rather than giving fixed target of salt and fluid intake, the advice is to alter to be more or less than the patient's current intake levels.	Usually used if urine volume less than 500 ml and start/increase dose before changing PD regime to achieve more daily fluid removal. Maximum dose is 100mg. Stopped when anuric.	First choice of antihypertensive agent where required, unless for economic reason as short acting calcium channel blocker and clonidine is cheaper option.	Not available

4.7 Discussion

The most important finding of this trial was the greater than anticipated stability over 12 months of BI derived fluid volumes, especially in non-anuric patients. Where significant changes were seen, with or without adjustment for baseline factors associated with BI volumes (gender, age, comorbidity), these were reductions in the TBW with or without parallel change in the ECW. In anuric subjects this was spontaneous and likely to reflect loss in lean tissue, as we originally hypothesised, leading to an increase in the ECW/TBW ratio. There was marginal evidence that this could be ameliorated in the Shanghai BI-intervention group who achieved greater stability of the BI vector associated with higher dialysate glucose prescription, but no difference in blood pressure which was well controlled in both groups. In the UK non-anuric BI-intervention group the fall in TBW was due to the setting of a lower target weight but no change in the ECW/TBW ratio or improvement in blood pressure. Taken together these findings suggest that longitudinal BI vector plotting adds little additional value to clinical fluid management.

The spontaneous fall in BI derived TBW with increase in ECW/TBW seen in the anuric patients is in keeping with our own study of longitudinal body composition in hemodialysis patients in which increased ECW/TBW associated with comorbidity reflected overhydration supported by independent measures of TBW from deuterium dilution (Chan, McIntyre et al. 2009) as well as longitudinal studies using various methods in peritoneal dialysis patients (Johansen, Kaysen et al. 2003). It is important to emphasise that our study design was intended to address this problem by showing how BI may be used to detect longitudinal changes in body composition and adjust for these rather than using it as a tool to drive patients to a specific target hydration status. This approach is in

contrast to other current (Su, Gangji et al. 2011, Liu, Long et al. 2012) or recently completed study protocols (Hur, Usta et al. 2013, Onofriescu, Hogas et al. 2014) and was chosen in order to minimise risk, especially premature loss of residual kidney function. Indeed a recent study using the overhydration index to reduce dry weight was associated with significant loss of urine output in non-anuric hemodialysis patients (Hur, Usta et al. 2013). This problem was not reported in the only other trial completed in PD patients that did find BI improved fluid status and blood pressure but follow up was limited to three months only (Luo, Lu et al. 2011). Our decision to use vector plots of BI data rather than calculated volumes is also worthy of comment. This was driven by a number of considerations. First, given the lack of a known value for optimal ECW volume in the PD population in which overhydration is driven by many factors such as comorbidity and hypoalbuminaemia we were reluctant to set absolute targets. This was mainly for the reasons already given, and the vector plot approach discourages clinicians from making such judgements. Second, this made it very easy to blind clinicians from the BI data which necessitates plotting before it can be interpreted and by delaying the calculations of interpolated volumes on which the study was powered until after the study data lock applied we were able to minimise the effect of clinician bias. Third, there are very large databases giving normal values for vectors in the general population (Bosy-Westphal, Danielzik et al. 2005) as well as the dialysis population in which both phase angle and vector length have been shown to be highly predictive of survival (Pillon, Piccoli et al. 2004). At the time of study design this data was not available for other methods, in particular the overhydration index. Finally the BI devices we used have a well established record in reproducibility and were relatively inexpensive. Of interest a similar approach has been adopted in the FLUID study protocol (Su, Gangji et al. 2011).

One of the purposes of our study was to capture information on decision making by clinicians in an attempt to see how the use of BI informs practice. Overall, most clinical assessments did not lead to a change in target weight and it should be recognised that spontaneous changes in weight without a change in hydration will have occurred in many patients, reflecting, for example, changes in body fat. In the control groups the decision to increase or decrease target weight was remarkably symmetric, whereas increasing the weight in the intervention groups was less likely. It is possible that for the UK clinicians the tendency to make greater weight reductions in the intervention group so as to achieve better blood pressure control was due to study bias. The types of intervention used, although recorded, proved difficult to analyse as these were usually multiple, i.e. of several types and repeated within patients and not demonstrably different between control and BI groups. The relative maintenance of urine volume, despite a reduction in target weight, could be explained by diuretics, as observed in a previous trial comparing diuretic use with placebo, (Medcalf, Harris et al. 2001) but this could not be clearly shown here due to the fact that most patients were already on diuretics and that most interventions were complex. In any case this did not translate into improvements in hydration status as judged by ECW volume, ECW/TBW, phase angle or blood pressure management. However this does support observational data indicating that preservation of residual urine volume does not depend on maintaining overhydration (McCafferty, Fan et al. 2014). There is a concern that the loss of weight might reflect a reduction in muscle mass, but the change in TBW was not associated with a significant change in the raw electrical data, e.g. resistance, and is therefore dominated by the weight change fed into the algorithm used to extrapolate TBW, and so might be due to loss in body fat. This complex effect deserves further evaluation and a better understanding of how patients respond to requests to reduce their target dry weight that should inform future trial design. The trend to reduce weight,

especially for the purpose of blood pressure control was also seen in the Shanghai patients but the absolute changes were not so great, perhaps reflecting the overall better blood pressure measurements in the Chinese patients. The value of BI in the anuric intervention group here appears to have been a better targeting of decision making combined with a significant increase in glucose prescription so preventing the drift towards worsening tissue oedema. This increase in glucose use will also have increased calorie intake so protecting against loss of lean tissue which was less severe in this group.

This study has a number of limitations. Although we went to great lengths to conceal allocation process and blind clinicians to the control BI data and outcomes, it was by definition impossible to avoid the knowledge of randomisation group and thus eliminate decision bias by clinicians. It may well be, for example, that the more aggressive weight reduction in the UK-non-anuric intervention group reflected this rather than specific information obtained from the BI measurements *per se*. Reasons not to be included in the study differed by country, being planned modality transfer, especially transplantation, in the UK versus greater likelihood of failing to achieve stability in the run-in period in Shanghai, which suggests that the Chinese anuric patients could be more selected. However it can equally be argued that this selection applied to the UK even more so given the greater difficulty in recruiting anuric patients. The unequal numbers in the UK randomisation which in retrospect should have been stratified by centre, did not affect the balance of measured baseline patient characteristics and the failure to recruit sufficient anuric patients from the UK, has been partially addressed by including a multivariate analysis incorporating both data from both countries. This analysis showed that age, gender and comorbidity are the main determinants of body composition and that any differences between centres were a function of patient level characteristics and interventions available.

More detail in describing the dietetic interventions would have been desirable, e.g. patient level data on salt intake as this turned out to be more important than was initially appreciated; we considered measuring sodium losses but these have previously been shown to be an unreliable indicator of dietary change in PD patients (Cheng and Wang 2006). The strengths of the study were its multicentre, pragmatic design which enhances its generalisability.

4.8 Conclusions

In conclusion, routine use of longitudinal BI to inform fluid management in PD patients had minimal impact over 12 months. Although this was partly because body composition is very stable in non-anuric patients it could be because we chose to maintain a stable BI vector rather than intervene more aggressively to normalise fluid status. Future studies are needed to evaluate such an approach but will require careful choice of clinically relevant outcomes such as residual kidney function and blood pressure control. Importantly this study demonstrates just how difficult it is to capture and interpret the complex interventions and practice patterns associated with fluid management and thus attribute cause and effect. An increase in glucose prescription was the only intervention associated with benefit.

Chapter 5 Bioimpedance, fluid management and longitudinal cardiac function: A secondary outcome analysis of single centre data from the UK-Shanghai BIA study

Chapter 5 Bioimpedance, fluid management and longitudinal cardiac function: A secondary outcome analysis of single centre data from the UK-Shanghai BIA study

5.1 Introduction

Patients with end stage renal disease (ESRD) on dialysis therapy have a much higher rate of cardiovascular death compared to the age and gender matched population without renal disease. Despite the progressive improvement in the death rate over the past two decades, it still accounts for approximately 27% of all cause mortality (Registry, 2014). Congestive heart failure (CHF) is common among dialysis population. It was found in 31% of ESRD patients commencing on dialysis therapy and 25% of patients without CHF at baseline showed evidence of CHF subsequently with a median survival of 36 months (Harnett, Foley et al. 1995). This cardiac syndrome is closely associated with LVH, IHD and systolic failure which all contribute to the high cardiovascular burden (Parfrey, Foley et al. 1996).

Beyond the usual conventional cardiac risk factors such as coronary artery disease, diabetes mellitus, hypertension and dyslipidaemia that dialysis patients need to deal with , they are also exposed to the renal or dialysis related negative cardiac predictors such as uraemia, anaemia, hypoalbuminaemia and inflammation. Fluid overload is increasing being recognised as a concerning cardiac risk factor in ESRD patients which predicts mortality independent of hypertension (Wizemann, Wabel et al. 2009, Agarwal 2010). The mechanism by which overhydration aggravates cardiovascular death is complex and multifaceted. PD patients with uncontrolled hypertension are more volume overloaded as

determined by BIA and antihypertensive medication alone is unlikely to be effective without salt and fluid restriction (Wang, Axelsson et al. 2005). PD patients with history of volume overload have more severe left ventricular hypertrophy and left ventricular dilatation and 80% have evidence of diastolic dysfunction with normal ejection fraction (Wang, Sanderson et al. 2003). The loss of residual renal function (RRF) with time on dialysis has been showed to be an independent predictor of survival both in PD (Bargman, Thorpe *et al.* 2001, Paniagua, Amato et al. 2002) and HD (Termorshuizen, Dekker et al. 2004) cohorts. This leads to inadequate sodium removal, thus compounding the problem of overhydration by increasing the incidence of hypertension and LVH in dialysis patients (Enia, Mallamaci et al. 2001, Khandelwal, Kothari et al. 2003).

PD enjoys similar if not better survival benefit compared with HD at least for the first 2 years of therapy but subsequent outcome has not been as good (Collins, Hao et al. 1999, Gokal, Figueras et al. 1999, Tanna, Vonesh et al. 2000). This was despite the common perception that PD was thought to be a better treatment option due to its continuous ultrafiltration with less cardiovascular haemodynamic upheaval. The mortality risk of patients with CHF treated with PD increased with time independent of the diabetic status in a two year observational study (Stack, Molony et al. 2003). This suggests a possible deleterious effect of suboptimal volume status on the progression of CHF in PD patients. The primary findings of the multicentre BIA study suggest a relatively stable body composition in patients with residual renal function. We want to explore the impact of fluid management using BIA on cardiac function in non anuric PD patients over the 12 month period.

5.2 Aims of the study

We hypothesized the use of BIA in the fluid management of PD patients leads to a significant reduction of target weight and a better preservation of cardiac function. We also wanted to examine the impact on the residual renal function.

5.3 Subjects and study design

This was a predefined sub-analysis of the BIA study. The patient population was derived from the multi-centre BIA study. (Developing Bioimpedance (BIA) as a tool for fluid management in Peritoneal Dialysis Patients: A validation Study ClinicalTrials.gov NO: NCT00801112.) Patients recruited from the University Hospital of North Staffordshire who had echocardiographic measurement as part of their baseline and final follow up visit were included in this sub-analysis.

Inclusion criteria were similar to the BIA validation study (see Chapter 4). Briefly, the eligible patients had to be 16 years old or above and had been established on PD for more than 3 months and deemed clinically stable. Patients with acute inflammation, e.g. peritonitis within one month or with life expectancy less than six months were excluded. Fluid status was determined and optimised clinically at baseline prior to study enrollment. Patients were excluded from the study if optimal fluid status, which was defined as the minimal body weight that patient can achieve without adverse effect, e.g., hypotension, could not be achieved by clinical means. Following optimisation of fluid status the patients were randomized into active and control groups. In the active group bioimpedance vector plots were used in combination with clinical assessment (BP, oedema) to manage fluid

status. In the control group, BIA measurements (resistance and reactance) were recorded but were not used in decision making.

5.4 Materials and methodology

5.4.1 Clinical measurements

Comorbidity was recorded at the time of enrollment by the consultant. Stoke comorbidity score were used in the study. In brief, 7 comorbidity domains were considered, including non-cutaneous malignancy, ischemic heart disease (IHD), peripheral vascular disease (PVD), left ventricular dysfunction (LVD), diabetes mellitus (DM), systemic collagen vascular disease, and any other condition thought to reduce life expectancy. The comorbidity score was the number of these domains affected. The comorbidity grade was then derived from the comorbidity score. Grade 0 (low risk) was a zero score, grade 1 (medium risk) was a score of 1-2, and grade 2 (high risk) a cumulative score of ≥ 3 .

Membrane function was evaluated by standard peritoneal equilibration test (2.27% PET). The clinical routine biochemistry profiles were measured as part of clinical practice. The sampling date of the biochemistry profile may or may not be on the same day as BIA measurement but the interval between measurements was kept less than a month.

5.4.2 BIA measurement

BIA parameters were measured by a single frequency (50Hz) BIA device (Akern BIA 101 Anniversary) in the study. The total body resistance and reactance were measured

according to the operator's manual for the further calculation of total body water (TBW) and extra-cellular water (ECW). (Appendix 2)

5.4.3 Echocardiographic measurements

Left ventricular mass and left atrial volume were determined from standard 2-dimensional echocardiography and corrected for body surface area (BSA). E/A ratio, a parameter for mitral inflow or diastolic function and E/E' ratio, a surrogate marker for left ventricular filling pressure were measured concurrently as described in chapter 2.

5.5 Statistical analysis

Continuous data were expressed as mean values \pm SD or median (inter quartile range), as appropriate. For data not normally distributed, Kruskal Wallis H test was applied to test the difference between groups. Unpaired t-test were applied to compare difference between two independent variables. Paired t-test was used to compare the difference between baseline and follow up data. Multi-level strategy was applied in the multi-variant analysis. P values <0.05 was considered to be statistically significant. Multi-variant analysis was done by MLwin. Other statistical analyses were performed using IBM SPSS software, version 20.0 (SPSS, Chicago, IL)

5.6 Results

5.6.1 Baseline patient characteristics: body composition and cardiac parameters

57 patients from University Hospital of North Staffordshire were recruited in the BIA study of which five anuric patients were excluded from this sub-analysis due to the limited number. Hence, this sub-analysis was only confined to the 52 non anuric patients.

Recruitment, randomization and dropout were summarized in the consort diagram in Figure 5.1 and no significant difference was found regarding dropout rate or reason between active and control group. More patients were randomised to the control compared to the active group (n=31 vs 21) and after accounting for dropouts 24 and 15 patients completed the study for final analysis respectively. Patients in the active group were significantly older than the control group (63.2 ± 13.7 vs 54.52 ± 15.8 years, $P=0.05$). Both groups showed similar Stoke comorbidity grades, dialysis vintage, residual renal function, blood pressure and body composition as determined by BIA measurements. The data are summarised in Table 5.1 and Table 5.2.

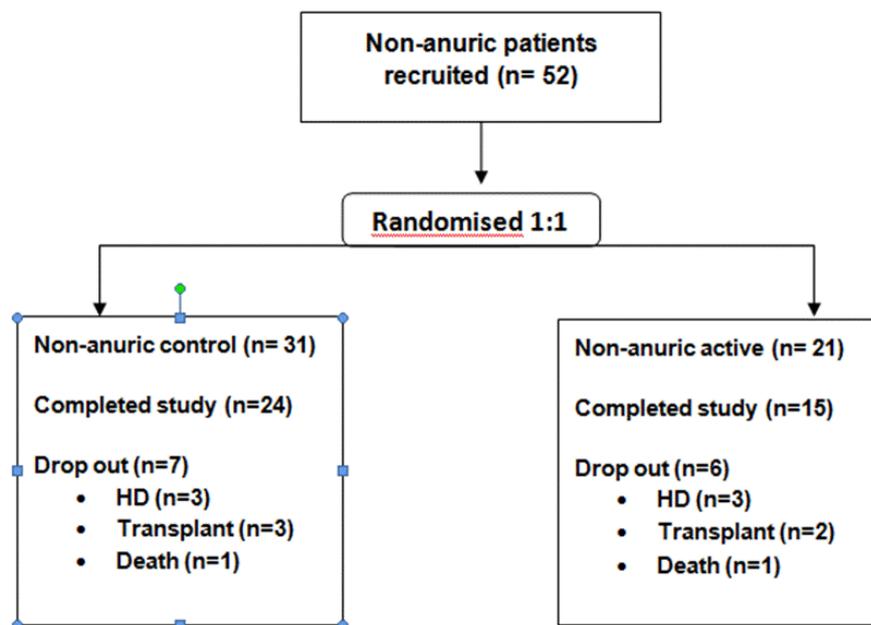


Figure 5.1 Consort diagram of recruitment, randomization and drop out

Table 5.1 Baseline clinical characteristics of active and control group

	Control	Active	P value
N	31	21	
Gender (female/male)	13/18	6/15	0.39
Age (year)	54.52±15.8	63.2±13.7	0.05
Time on PD (mon)	25.26±23.8	22.9±20.1	0.71
Urine volume (ml)	1,512±832	1,228±633	0.20
Renal Kt/V	1.28±0.8	1.0±0.6	0.28
Albumin(g/L)	33.1±4.2	32.0±5.5	0.46
Systolic BP(mmHg)	154.8±22.6	144.7±21.7	0.13
Diastolic BP (mmHg)	86.4±12.2	79.2±15.2	0.08
Clinical weight (kg)	78.9±20.1	82.7±12.5	0.41
Dialysate dose (ml)	8,560±1,932	8,920±2,067	0.53
Total Glucose(g)	118.9±44.2	114.1±39.1	0.71
Average Glucose (g)	1.37±0.3	1.4±0.2	0.82
TBW(L)	40.86±8.2	44.0±7.0	0.16
ECW (L)	18.73±4.0	20.3±3.4	0.14
ECW/TBW	0.46±0.1	0.5±0.1	0.76
Height ² /Resistance(cm ² /Ω)	56.55±12.0	61.9±12.0	0.12
Height ² /Reactance(cm ² /Ω)	551.06±149.9	614.3±156.8	0.15

Table 5.2 Stroke comorbidity grade in randomization group

Stroke Comorbidity Grade	Control	Active	Total
0	13	7	20
1	15	12	27
2	3	2	5
Total	31	21	52

Left ventricular mass index and ejection fraction were comparable in both control and active groups. However E/E' was significantly higher in the active compared with control group at baseline (11.9 ± 6.0 vs 8.95 ± 2.9 , $P=0.05$). Echocardiographic tissue velocity in systolic and diastolic phases were similar in both groups. Cardiac data were summarised in Table 5.3.

Table 5.3 Baseline echocardiograph characteristics of active and control group

	Control	Active	P value
N	31	21	
Left Vent. Mass Index (g/m ²)	114.78±38.6	116.3±37.2	0.89
Left Atrial Volume Index (ml/m ²)	32.31±10.5	36.4±12.9	0.22
LV Ejection Fraction (biplane, %)	54.39±9.8	49.9±11.1	0.14
Stroke Volume (VTI, ml)	67.71±24.8	79.2±20.5	0.09
Cardiac Output (l/min)	4.61±1.6	5.2±1.4	0.17
E/E'	8.95±2.9	11.9±6.0	0.05
Systolic TV (septal/lateral walls) cm/s	5.61±1.6	5.4±1.5	0.62
Early diastolic TV (cm/s)	5.71±2.4	4.7±2.4	0.13
Late diastolic (atrial) TV (cm/s)	6.66±1.9	7.2±1.8	0.29
Systolic TV (4 walls) cm/s	5.53±2.5	4.7±2.4	0.24

TV - tissue velocity

5.6.2 Longitudinal change in active and control group: Body composition and cardiac parameters

Taken as a whole, patients from both study groups demonstrated progressive worsening of LVEF (-2.8%, 95% CI -5.6 – -0.025, P<0.05), E/E' (+1.3, 95% CI -0.43 – 2.1, P<0.05) and

systolic wall tissue velocity (four walls) (-0.46, 95% CI -0.11 – -0.81, $P<0.01$) (see Table 5.4). The paired t-test showed a significant decrease in LVEF in controls over 12 months (56.9 ± 6.1 vs 52.5 ± 6.5 , $P=0.013$), whereas change in the active group was non-significant (54.0 ± 7.4 vs 52.3 ± 5.2 , $P=0.33$). E/E' worsened in control group (8.6 ± 2.3 vs 10.8 ± 2.8 , $P<0.01$) but no change was observed in active group (11.0 ± 3.2 vs 11.5 ± 2.6 , $P=0.416$) (see Table 5.5). E/E' was associated with ECW/TBW but showed inverse relationship with RRF, both at baseline and 12 month. Target weight was more likely to be reduced in the active group (-3.3kg vs -0.05kg, $P<0.05$) resulting in greater decrease in clinic weight compared with the control group (-3.0kg vs -2.6kg, $P<0.05$). No significant change in ECW/TBW ratio was observed in both groups longitudinally (+0.012 in control vs +0.02 in active, $P=NS$). Urine volume was better maintained in the active group but no between group differences was found in residual renal clearance or glucose exposure (see Table 5.6). On multivariate analysis LVEF and E/E' at 12 months were independently predicted by their respective baseline values and study randomisation group after controlling for comorbidity status, age, gender and baseline BIA (Table 5.7 and Table 5.8). Decision to reduce target weight was more likely to be made in active group than in control group (Table 5.9).

Table 5.4 Longitudinal cardiac patients for all patients from both groups combined

	change	95% CI
Left Vent. Mass Index (g/m ²)	+2.3	(-4.7, 11.8)
Left Atrial Volume Index (ml/m ²)	+2.8	(-2, 6.9)
LV Ejection Fraction (biplane, %)	-2.8	(-5.6, -0.025)
Stroke Volume (VTI, ml)	-4.6	(-12.7, 3.5)
Cardiac Output (l/min)	-0.23	(-0.7, -0.001)
E/E'	+1.3**	(0.43, 2.1)
Systolic tissue velocity (TV) (septal/lateral walls) cm/s	-0.37*	(-0.73, -0.001)
Early diastolic TV (cm/s)	+0.06	(-0.4, 0.5)
Late diastolic (atrial) TV (cm/s)	-0.23	(-0.63, 0.17)
Systolic tissue velocity (4 walls) cm/s	-0.46**	(-0.11, -0.81)

*, P<0.05; **, P<0.01; †, P=0.05

Table 5.5 Longitudinal change of echocardiograph characteristics in active and control group

	control	active
N	24	15
Left Vent. Mass Index (g/m ²)	-0.96 (-15,+14)	+14 (-4.5, +33)
Left Atrial Volume Index (ml/m ²)	+4.2 (-1.1, +9.5)	+2.5 (-7.5, +12)
LV Ejection Fraction (biplane, %)	-4.4 (-1, -7.8)*	-1.6 (-5.3, +1.9)
Stroke Volume (VTI, ml)	-3 (-15, +9)	-7.6 (-27, +12)
Cardiac Output (l/min)	-0.12 (-0.8, +0.6)	-0.27 (-1.3, +0.8)
E/E'	+2.3 (1.2, 3.3)**	-0.45 (-0.7, +1.6)
Systolic tissue velocity (TV) (septal/lateral walls) cm/s	-0.47 (-0.02, -0.93)*	-0.17 (-0.86, +0.52)
Early diastolic TV (cm/s)	-0.11(-0.6, +0.4)	+0.18 (-1, +1.3)
Late diastolic (atrial) TV (cm/s)	-0.08 (-0.6, +0.45)	-0.61 (-1.6, +0.37)
Systolic tissue velocity (4 walls) cm/s	-0.19 (-0.74, +0.34)	-0.24 (-0.8, +0.35)

*, P<0.05; **, P<0.01

Table 5.6 Longitudinal change of patient characteristics in active and control group

	control	active
N	24	15
Target weight (kg)	-0.05 (-1.7,1.6)	-3.3 (-0.7, -5.8)*
Clinic weight (kg)	-2.6 (+2.3, -7.5)	-3.0 (-0.33, -5.6)*
Urine volume (ml)	-405 (-139, -762)**	-149 (-442, +144)
Renal Kt/V	-0.3 (-0.5, -0.55)*	-0.24 (-0.4, +0.05)*
Systolic BP	-12 (-2, -21)*	-3.5 (-11, +18)
Diastolic BP	-4.9 (+0.1, -9.8)†	-0.5(-9, +8)
Albumin (g/l)	-0.11 (-0.03, +0.01)	+0.11 (-0.05, +0.26)
Daily UF (ml)	167(-43, 376)	-41(-265, 183)
Total dialysis glucose (g/day)	+3.8 (-20, +27)	+6.6(-12, +25)
ECW (L)	+0.64 (-0.7, +2.0)	+0.3 (-1.8, 2.5)
TBW (L)	+0.36 (-0.9, +1.6)	-1.58 (-3.1, +0.01)†
ECW:TBW ratio	+0.012 (-0.01, +0.04)	+0.02 (-0.02, +0.06)
Hight ² /Resistance(cm ² /Ω)	1.09(-1.77, 3.94)	-2.56(-6.21, 1.09)
Hight ² /Reactance(cm ² /Ω)	45.59(-33.49, 124.67)	24.57(-89.43, 138.57)

*, P<0.05; **, P<0.01; †, P=0.05

Table 5.7 Multivariate modelling of longitudinal change of echocardiograph parameters for LVEF.

	model for LV EF		
	β	standard error	P value
Constant	52.73	1.88	<0.01
LV EF (Baseline)	0.41	0.12	<0.05
Comorbidity	-2.91	1.39	<0.05
Gender	-1.59	1.67	NS
Age	-0.16	0.06	<0.05
Active (compare with control)	4.44	1.76	<0.05
ECW/TBW (Baseline)	-5.04	15.09	NS

Table 5.8 Multivariate modelling of longitudinal change of echocardiograph parameters for E/E'

	model for E/E'		
	β	standard error	P value
Constant	11.32	0.73	<0.01
E/E' (Baseline)	0.62	0.12	<0.01
Comorbidity	0.60	0.58	NS
Gender	-0.23	0.69	NS
Age	0.03	0.03	NS
Active (compare with control)	-1.47	0.76	0.05
ECW/TBW (Baseline)	7.88	6.29	NS

Table 5.9 Clinical decision in active and control group

	Target decrease	Target unchanged	Target increase
Control	19	85	18
Active	17	64	5
Chi square P value			<0.05

5.8 Discussion

In this single centre secondary analysis of the UK-Shanghai BIA study it was observed that non anuric PD patients with BIA guided fluid management had better preservation of residual renal function and echocardiographic cardiac parameters namely the left ventricular ejection fraction and the early mitral inflow velocity to peak mitral annulus velocity (E/E') ratio. However, the incorporation of BIA information in the clinical assessment of fluid status had very little impact on body composition as determined by BIA despite the decision to reduce target weight being more likely to be made in this group. The finding of stable fluid status over the 12-month study period in the non-anuric PD patients was consistent with the result of the main study.

It is important to state that over time patients in both study group showed progressive deterioration in LVEF and E/E'. The multivariate analysis indicated that the most important predictors of cardiac function at follow-up were their base line cardiac function and comorbidity as well as randomisation group. Baseline fluid status (ECW/TBW) was not a predictor. Although we found, probably for the first time, that better preservation of both of the echocardiographic parameters was seen in the active BIA group, the reason for such findings are not immediately apparent. Fluid status was not different in these two groups despite the intervention to reduce weight, but strikingly, and perhaps surprisingly RRF was better preserved in the BIA group. RRF had been showed to correlate with LVH and LVEF in PD patients (Wang, Wang et al. 2002) and regression of LVH was observed in ESRD patients who had successful kidney transplant (Parfrey, Harnett et al. 1995, Rigatto, Foley et al. 2000). One could speculate better clearance of uraemic toxic with higher urine output and this may have slowed down the deterioration of left ventricular

systolic function. We did not measure the central or intravascular volume in this cohort that could have more impact on RRF but we do not expect any significant difference given our previous study has revealed plasma volume in PD patients was not expanded (see Chapter 2). It is possible, therefore, that BIA directed therapy resulted in interventions that protected loss of RRF; however it was not explained by increased diuretic prescription.

The use of early mitral inflow velocity to peak mitral annulus velocity (E/E') ratio by tissue doppler imaging has been validated as a surrogate parameter of elevated left ventricular filling pressure (LVFP) and is a powerful predictor of survival in cardiac patients with systemic hypertension and left ventricular systolic dysfunction (Wang, Yip et al. 2005, Wang, Yip et al. 2005). Studies in a PD population revealed up to 60% of patients had evidence of elevated E/E' which added significant prognostic value for all cause mortality and cardiovascular death (Wang, Wang et al. 2008). Moreover, loss of RRF was found to be a significant factor associated with increased E/E' in the same study. A recent study in ESRD patients indicated an E/E' ratio of 15 or more has a sensitivity of 82% and specificity of 88% in predicting a LVEDP of 15 mmHg or more which, in turn predicts mortality (Sharma, Pellerin et al. 2006). The mean E/E' ratio in our study was 10.45 ± 5.0 (8.95 ± 2.9 in control and 11.9 ± 6.0 in active group) which was less than the threshold value reported in the study by Sharma et al and significantly much lower than reported in the Chinese PD population by Wang et al (20.1 ± 9.7) (Wang, Wang et al. 2008). Such magnitude of discrepancy may indicate different ethnicity in the study population with varying body composition, preservation of residual kidney function and cardiac function or different clinical practice or a combination of these factors.

There are several limitations in our study which was only designed as being exploratory and not powered to the predefined outcomes. This is a small single centre study with considerably fewer patients randomised to the active group. This randomization imbalance arose from the failure to stratify by centre. We did not include anuric patients in the analysis considering the small number of such patients in our population. The problem with older patients with higher E/E' ratio in the active group has been discussed.

5.9 Conclusions

In conclusion, we found that cardiac function declines over time on PD in particular LVEF, E/E' ratio and systolic wall motion. The clinical significance of these changes is unclear and warrants further investigation. The use of BIA has led to a systematic reduction in target weight, better preservation of urine volume and less deterioration in cardiac function. The mechanism of this benefit, which might be subject to Type 1 error, is unclear. Further studies to evaluate the use of BIA to target fluid management with the hope of protecting both the heart and residual kidney function are desirable.

Chapter 6 Dispersal kinetics of deuterated water in the lungs and airways following mouth inhalation; real-time breath analysis by flowing afterglow mass spectrometry (FA-MS)

6 Chapter 6 Dispersal kinetics of deuterated water in the lungs and airways following mouth inhalation; an exploratory real-time breath analysis by flowing afterglow mass spectrometry (FA-MS)

6.1 Introduction

The lungs are relatively dry organs and complex mechanisms exist to maintain this state to facilitate gas exchange across the alveolar interface that separates the blood from the lung air. Accumulation of fluid in the lungs (pulmonary oedema) is a common manifestation of many illnesses ranging from severe infection to heart or kidney failure. The development of pulmonary oedema is an insidious process and the clinical signs such as the presence of raised jugular venous pressure, chest crepitation on auscultation and peripheral oedema often present late. The routine use of chest radiograph is too insensitive to discriminate pulmonary oedema from other lung pathologies (Sivak, Richmond et al. 1983, Eisenberg, Hansbrough et al. 1987). Currently, there is a lack of a suitable clinical tool to aid clinicians in the management of this condition.

The gold standard method for measuring extravascular lung water volume, EVLW, is the post-mortem gravimetric method (Pearce, Yamashita et al. 1965). This *ex-vivo* method is clearly of no use for clinical purposes. There are other techniques that evaluate EVLW such as computer tomography (CT), positron emission tomography (PET) and nuclear magnetic resonance (NMR) imaging. These have been validated against gravimetric

measurement providing quantitative estimation of EVLW (Meyer, Schober et al. 1984, Cutillo, Morris et al. 1986, Lange and Schuster 1999). Unfortunately, they are cumbersome, expensive, involve radiation and require specialist input. These shortcomings severely limit their general application outside the research setting.

The dilution method using single or double indicators is another technique that is frequently used at the bedside. This method is based on mathematical concepts that enable calculation of the volume of distribution of an indicator injected into the circulation (Isakow and Schuster 2006). This requires the presence of a central venous access for indicator injection and a detector in the arterial system commonly inserted in the femoral artery. Due to its invasive nature, this technique is only employed in the intensive care setting where the monitoring of haemodynamic parameters is crucial to guide therapeutic intervention. This precludes its use in outpatient setting such as for patients with renal failure attending for haemodialysis treatment or heart failure monitoring.

Deuterium labelling was used as early as the 1950's to investigate the exchange of water molecules across pulmonary capillaries (Lilienfield, Freis et al. 1955). However, early techniques for measuring deuterium concentration, such as the spectrophotometric method, were labour intensive and the results were not immediately available to the clinician. Wallin et al has showed experimentally that patients with renal failure undergoing haemodialysis are more likely to have subclinical pulmonary oedema compared with healthy controls (Wallin, Jacobson et al. 1996). In this study, a double indicator dilution densitometric technique was employed using D₂O and Indocyanine Green (ICG), a non-diffusible indicator. A mixture of the two was administered via the venous limb of the

arterio-venous fistula which is a readily available vascular access, and blood was sampled in the arterial limb. By studying the cardiac output and mean transit time from the dilution curves of the indicators, it is possible to deduce the lung water volume. However, real time measurement is not possible and specialised equipment is needed to detect ICG.

Flowing afterglow mass spectrometry (FA-MS) is a relatively new technique that offers on-line, real time determination of deuterium abundance in water vapour in exhaled breath and in the headspace of donated fluid such as blood and urine. It does not require sample preparation and with a typical precision and accuracy of 1% it is a powerful tool in the study of body composition, in particular diseased states (Smith and Spanel 2001, Spanel and Smith 2001). So far, it has been used to measure total body water (TBW) by breath analysis following the ingestion of known quantity of D₂O and to study the characteristics of water transport across the peritoneal membrane in patient treated by continuous ambulatory peritoneal dialysis (CAPD) (Davies, Spanel et al. 2001, Smith and Spanel 2001, Asghar, Diskin et al. 2003). We now propose FA-MS may be of use in the study of lung water measurement.

6.2 Aim of the study

The main hypothesis is that following the delivery of a known quantity of deuterated water vapour, D₂O, to the pulmonary airway, it rapidly equilibrates with lung water to form HDO. Then, by determining the exhaled breath HDO concentration and adopting the principle of isotope dilution (Meier and Zierler 1954), lung water volume can be estimated. The purpose of this preliminary pilot study is to observe the dispersal kinetic of HDO in the major pulmonary airway and alveoli following the inhalation of D₂O vapour.

6.3 Study subjects and methodology

Three healthy adult volunteers gave informed consent and were recruited for this limited pilot study. All three are non smokers and have no known lung pathology. 1 ml of neat D₂O liquid (99.9% pure, Cambridge Isotope Laboratories, USA) was injected into a Tedlar bag inflated with clean, dry cylinder air to a volume of about 5 litres where the D₂O vapour reached its saturated vapour pressure at room temperature – about 2 kPa. Baseline measurements of breath HDO abundance were then performed using FA-MS following which the three subjects were asked to inhale deuterium from essentially identical bag preparations just once at maximum inspiration. This was followed by immediate sampling of the exhaled breath by FA-MS to determine the HDO abundance in sequential single exhalations. These exhalations continued until the HDO abundance reached the pre-ingestion baseline level. The measurements were carried out as the subjects sat upright adjacent to the FA-MS instrument, exhaling into a wide bore disposable cardboard tube having minimum resistance given by the standard breath sampling T-piece. The exhaled breath was sampled into the instrument via a heated capillary maintained at about 100°C. The details of the FA-MS analytical technique have been described in Chapter 2.

6.4 Results

Typical breath time profiles of HDO abundance before and after D₂O vapour inhalation are shown in Figure 6.1 and Figure 6.2, plotted as the signal levels, ion counts per second, c/s, of the m/z 74 and m/z 75 analytical ions (see FA-MS section in Chapter 2). These data provide the D/H levels that are shown in Figure 6.3 (a) and (b). The first part of Figure 6.1 shows several exhalations profiles prior to D₂O vapour inhalation. These provide the baseline D/H value in the exhaled water vapour, which is typically 160 parts-per-million,

ppm, of the exhaled water vapour. As can be seen, the D/H value is much greater in the first exhalation following the inhalation of the D₂O vapour, and then there is a steady decrease towards the pre-inhalation baseline level (see Figure 6.1). The high values of D/H in the early phase of the experiment in all subjects is largely due to HDO rich air contained in the mouth and trapped/released from the saliva. We observed small differences in the peak HDO abundance for the three volunteers and a rapid decay in the exhaled breath D/H value for all three volunteers, especially rapid for the healthy female A (Figure 6.3-a).

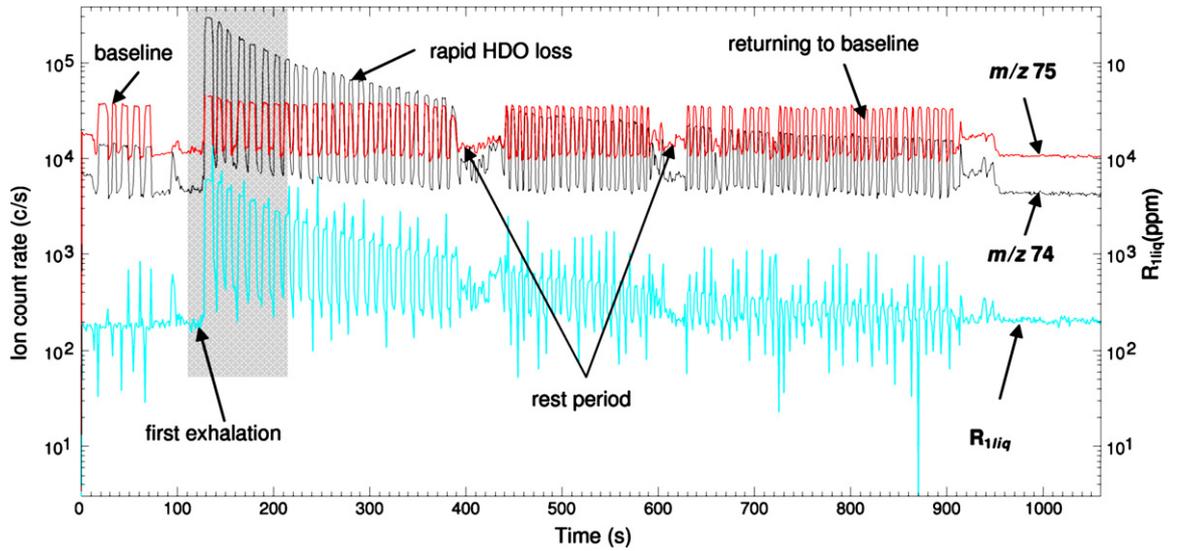


Figure 6.1 Multi ion profile provided by the FA-MS analysis software given as counts-per-second, c/s (left axis), of the analytical ions indicated.

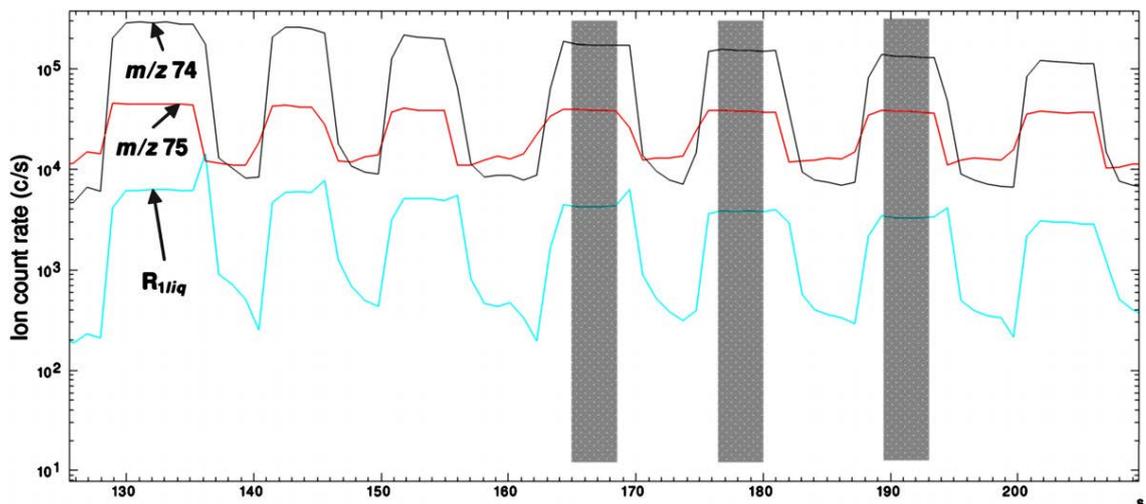


Figure 6.2 Expanded view of the shaded area in Figure 6.1.

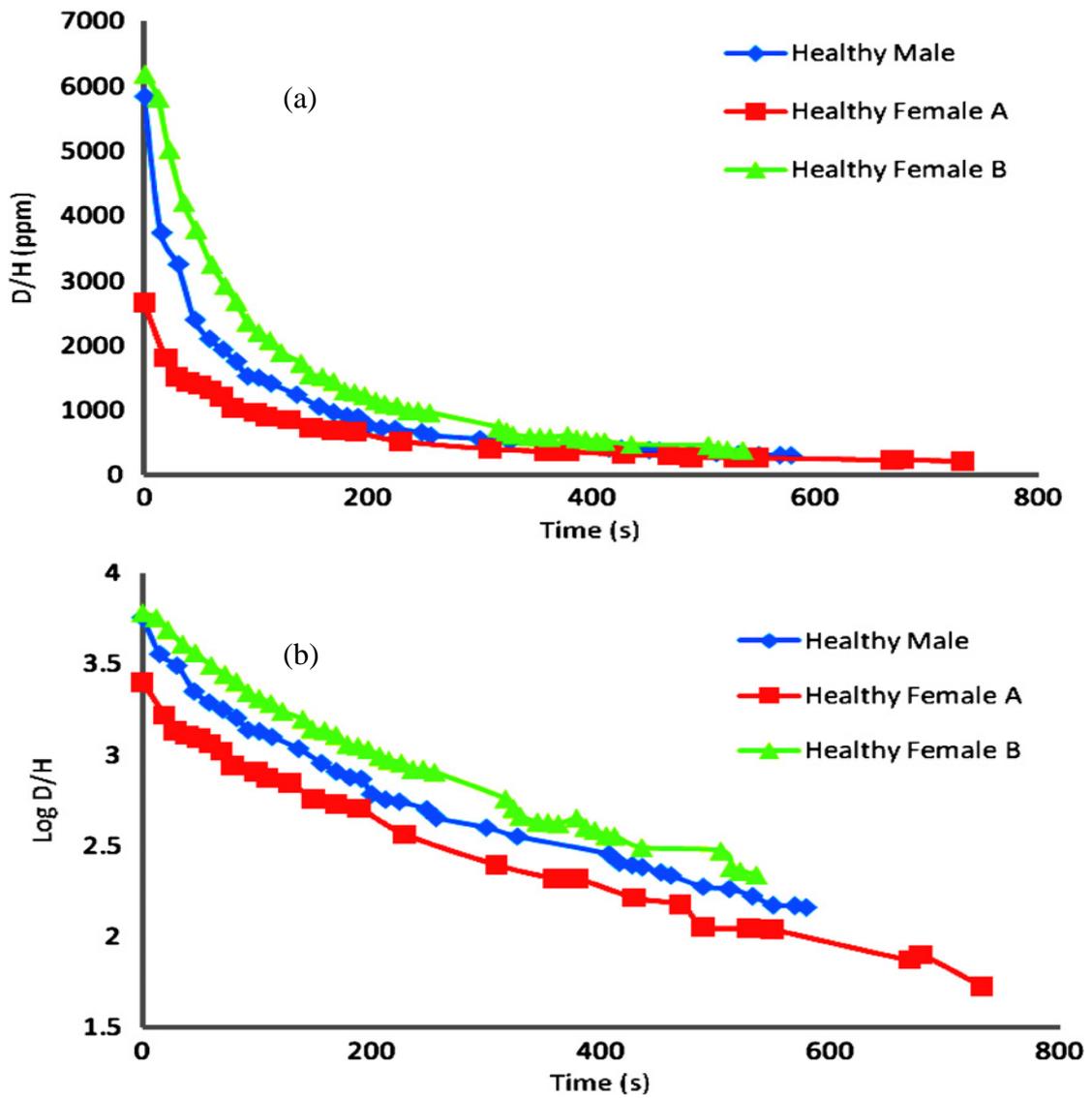


Figure 6.3 (a) The rapid decay of the breath HDO abundance following D_2O vapour inhalation. There is variation in the peak breath HDO level probably due to the differing inhalation techniques and lung capacities. (b) Log-transformed HDO abundance plotted against time; note the similar forms of the three decay curves.

6.6 Discussion

This preliminary study was intended to pave the way towards a rapid, non-invasive measurement of lung water volume and the monitoring of pulmonary oedema. The inhalation of a given amount of D_2O followed by the immediate measurement of the level of deuterated water in exhaled breath and applying the principle of isotope dilution provides a value for the lung water. Ideally, all the D_2O must pass into the lungs, fully mix with the alveolar water, then liquid water/vapour phase equilibrium must be quickly established before the vapour is exhaled along the airways without modification, then to be measured immediately using FA-MS. However it must be expected that rapid D/H exchange will occur when D_2O vapour contacts the liquid H_2O (mucosa) that lines the airway entry to the lungs, and so partial conversion of the D_2O to HDO will occur. Furthermore, it is likely that a fraction of the D_2O and the newly produced HDO will be absorbed into the airway mucosa and only an indeterminate fraction will reach the lung water in the alveolar space where the desired dilution will occur. Then, on the exhalation cycle, further dilution may occur when the HDO again makes contact with the airways mucosa. Finally, exhaled water vapour enriched with HDO emerges. The simplest aspect of this is the immediate determination of the exhaled breath D/H level using FA-MS. These phenomena have been described in some detail for a variety of trace compounds in inhaled and exhaled air (Anderson, Babb et al. 2003).

Complex as this scenario is, the data given in Figure 6.3 may provide some confidence in the pursuit of lung water measurement. The measured peak D/H values range from 2,600 ppm to 6,200 ppm for the inhalation of about 0.1 mL of D_2O (this corresponds to 4 L of inhaled air containing 3% of D_2O vapour). Given none of the absorption effects mentioned

above, this would equate to an effective water exchange volume of 16 mL to 37 mL. On this basis, it would appear that a great loss of D₂O/HDO does not occur and perhaps the peak D/H value is providing a sensible approximation to the volume of readily available water in the airways involved in mixing with inhaled vapour. The reason for the small variation in the peak D/H levels most probably lies in differences in the inhaled amount of D₂O, breathing rate and lung volume capacity, but given that lung water volume is quite similar in healthy individuals it is probable that this variation is due to the first two variables. These are the important issues that need to be addressed to take this technique forward when more control is introduced into the measurements.

The rapid decay of the exhaled breath D/H indicated by the data is very likely due to the absorption of the HDO into the total body water via transmission/mixing across the airways and, especially, flow into the blood (then body water) and due to exchange with water present in the inhaled ambient air. The similarity of the decay curves and the derived decay time constants is presumably because the HDO transmission rates in healthy individuals are similar. It may be that in pulmonary oedema, where the alveoli are partially blocked, the decay rate may be discernibly slower – a valuable medical parameter if shown to be the case.

There are several limitations of this study, these including the small number of healthy participants and no inclusion of patients. As mentioned above, no strict control was placed on the inhalation procedure, which should be easy to control for healthy people, but this might be more challenging for sick patients. The amount of D₂O/air inhaled was not

varied, but by doing so it might provide insight into the role of mucosa absorption, as mentioned above.

6.7 Conclusions

Pulmonary oedema is the worst spectrum of fluid overload characterized by abnormal accumulation of fluid in the alveolar space. The current gold standard bedside measurement of EVLW is the dilution method using various indicators but required invasive instrumentation. In this preliminary experiments, the dispersal kinetics of deuterium in exhaled breath of healthy participants following the inhalation of D₂O vapour are described. The basic idea of lung water estimation and future work using this novel technique is discussed.

Chapter 7 Injection of deuterated water into the pulmonary/alveolar circulation; measurement of HDO in exhaled breath and implications for breath analysis

7 Chapter 7 Injection of deuterated water into the pulmonary/alveolar circulation; measurement of HDO in exhaled breath and implications for breath analysis

7.1 Introduction

Patients with severe renal impairment, especially those with end stage renal failure dependent on renal replacement therapy have extraordinary high risk of cardiovascular morbidity and mortality. Salt and water homeostasis is severely disrupted and fluid overload is increasingly being recognised as the major obstacle in the effort to reduce cardiac disease burden. Patients with little or no urine output on dialysis therapy are critically dependent on the ultrafiltration function to remove enough fluid to avoid the detrimental effect of hypervolaemia. Patients with abnormal fluid status may exhibit little signs or symptoms to alert the clinicians as most have imperceptible tissue oedema in the internal viscera and organs. While subcutaneous oedema detected in the peripheral limbs causes minor inconvenience to the patients, it is often a late sign and cannot be relied upon for early detection and intervention for abnormal fluid status. One of the crucial and susceptible organs of such issue is the lung. The pulmonary system is a relatively anhydrous organ and for a good reason. Effective gaseous exchange simply could not take place if such environment is compromised, causing a state of hypoxia and progressive organ dysfunction with life threatening consequences.

Not surprisingly dialysis patients are prone to the development of pulmonary oedema, a medical condition characterised by abnormal accumulation of fluid in the extravascular space in the alveoli. Effective oxygenation is impaired and this leads to significant short-

and long-term morbidity and mortality. Therefore the detection and monitoring of pulmonary oedema by measuring lung water volume is crucial in the initiation and guidance of therapeutic intervention. The current gold standard bedside measurement of extravascular lung water volume (EVLW) are the dilution methods using various indicators, but despite the good correlation of the results with those obtained using the post-mortem gravimetric method, the invasiveness of the dilution technique limits its general application in the wider clinical setting.

The dispersal kinetics of deuterated water in the lungs and airways following mouth inhalation with real-time breath analysis using flowing afterglow mass spectrometry (FAMS) have been characterized in Chapter 6 in the bid to evaluate the feasibility of measuring lung water volume. This particular study has provided some insights regarding the phenomenon of absorption of HDO into the airways and predictably how the inhalation approach to determine lung water volume was compromised by D/H exchange between HDO and H₂O in the airways in both the inhalation and exhalation phases.

There is another equally important parallel objective to this study. It has been generally assumed that the concentrations of endogenous trace gas metabolites present in exhaled breath are representative of their concentrations in the general blood/alveolar circulation and thus, for many years, this premise has driven breath analysis research aimed at clinical diagnosis of particular diseases and for physiological study (Miekisch, Kischkel et al. 2008, Spanel and Smith 2011). However there has been some consideration given to the relationship between concentrations of breath metabolite and their concentrations in different parts of the respiratory system recently, firstly by Anderson et al (Anderson, Babb

et al. 2003), and then by King and his colleagues (King, Unterkofler et al. 2011, King, Unterkofler et al. 2012) using multi-compartment mathematical models that are compared with breath concentrations seen during exercise and for varying breathing patterns. Special attention was given to acetone, a highly soluble metabolite (King, Unterkofler et al. 2011, King, Unterkofler et al. 2012), and isoprene that is much less soluble, both being present in the exhaled breath of most humans (Salerno-Kennedy and Cashman 2005, Smith, Spanel et al. 2010). The relation of breath concentrations of isoprene and acetone to their arterial and venous blood concentrations had also been previously studied experimentally (O'Hara, Clutton-Brock et al. 2009).

The general finding of these modelling/experimental studies is that those water soluble metabolites, e.g. Acetone, released at the alveolar interface and appeared in exhaled breath are subject to efficient exchange at the surfaces of the various parts of the airways. If the steady state concentrations within the fluid lining of the airways are disturbed from those in the systemic circulation, exchange can take place as the air/acetone passes along the bronchial tree and the exhaled concentrations can differ from the alveolar (O'Hara, O'Hehir et al. 2008, Storer, Dummer et al. 2011, King, Unterkofler et al. 2012). Low solubility isoprene is a different case; it is established that this compound is stored in the major muscle groups of the body and is released into the systemic circulation during exercise (Koc, King et al. 2011) where the concentration may increase initially and then decrease as the store is depleted. Given isoprene exchange between the exhaled breath and the airways is not as efficient as for acetone, the exhaled breath concentration is a better reflection in the blood concentration. Similar basic ideas may be applicable to other metabolites, including the abundant carbon dioxide present in exhaled breath (Brewer, Orr et al. 2011), and this phenomenon throws into question the concept of an alveolar plateau (Meyer, Mohr

et al. 1990), which assumes that carbon dioxide is entirely released at the alveolar interface. It is obvious that exhaled carbon dioxide increases above that to the resting state during exercise but such increment is not observed in the exhaled acetone, whilst the carbon dioxide concentration rises from about 4% to 6% (Smith, Pysanenko et al. 2009). This can be explained by the fact that acetone is an entirely systemic compound in equilibrium amongst the blood/exhaled breath/urine, between which phases it readily exchanges, and the production of this compound is not significantly altered by moderate exercise (Pysanenko, Wang et al. 2009). This also throws into question the idea that breath concentrations of trace gas metabolites should be controlled or normalised against the concomitantly measured carbon dioxide concentrations (Schubert, Spittler et al. 2001, Liu and Pawliszyn 2007).

It is also relevant to mention that mouth-exhaled breath is known to be contaminated by trace compounds generated in the oral cavity and the airways. It is well observed that mouth-ingested exogenous compounds can be partially trapped and retained in the saliva and their release can contaminate the mouth-exhaled breath for period of minutes until the salivary circulation effectively removes them. This is best illustrated graphically in the study of using mouth ingestion of D₂O for the measurement of total body water (TBW) in renal patients and the analysis of the exhaled breath deuterium content using the principle of isotope dilution in Figure 7.1 (Davies, Spanel et al. 2001). The initial steep rise and fall of the D/H ratio is due to the contamination/decontamination of the saliva, with the peak value of the HDO concentration being inordinately high; typically, 60 to 90 minutes need to pass before the equilibrium concentration of HDO in the exhaled breath is achieved, which reflects equilibration with TBW.

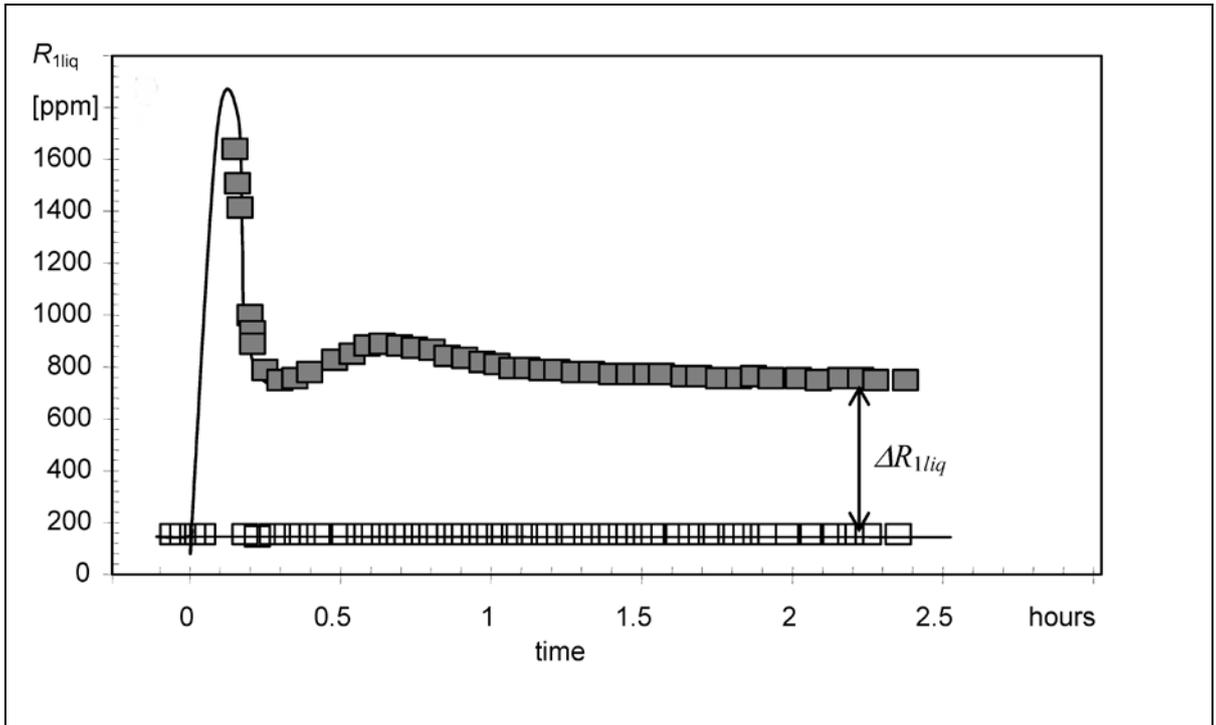


Figure 7.1 An example of a typical time variation of R_{1liq} in parts-per-million, ppm (filled squares) determined from breath HDO measurements using FA-MS following oral ingestion of 18.7 g of D_2O by a volunteer. The open squares are simultaneous measurements for a control who has not ingested D_2O (data from (Smith and Spanel 2001)).

7.2 Aims of the study

In the continuation of the challenge of lung water measurement, we chose to investigate another approach involving the injection of a known amount of an HDO/ H_2O mixture directly into the pulmonary circulation. We also seek to investigate the effects of HDO concentration in the exhaled breath as it travels through the pulmonary circulation and into the alveolar space before finally being sampled and measured by FA-MS (

Figure 7.2).

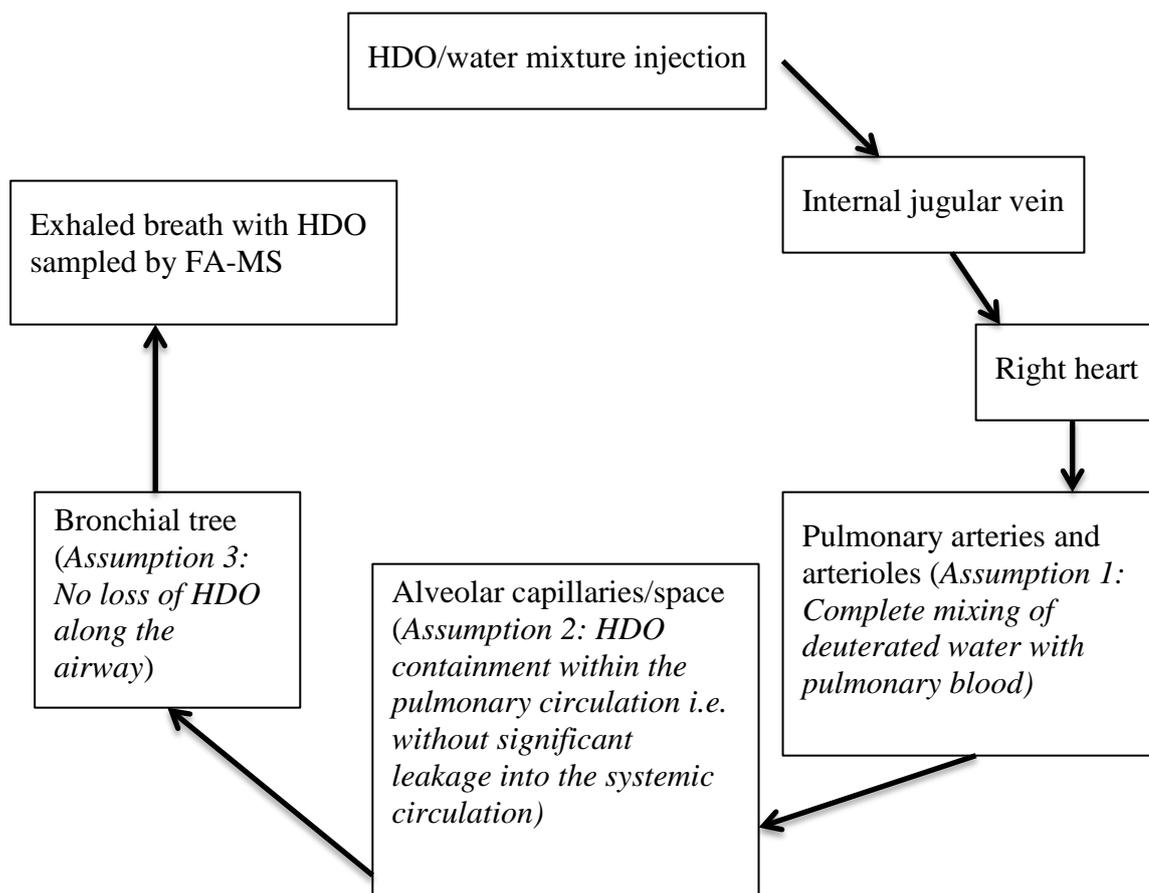


Figure 7.2 Flow chart demonstrating the pathway of HDO/water mixture through the cardio-pulmonary circulation following injection into the internal jugular vein prior to FA-MS sampling of the exhaled breath. The theoretical assumptions underpin the methodology are included.

7.3 Study subjects and methodology

7.3.1 FA-MS: Determination of the D/H content of water vapour

The details of the FA-MS analytical technique have been discussed before. Briefly, a swarm of H_3O^+ precursor ions is generated by a microwave discharge in flowing helium carrier gas possessing a trace of water vapour. These precursor ions react with the H_2O , HDO, H_2^{17}O and H_2^{18}O molecules in the water vapour contained in a breath sample (or the headspace of an aqueous liquid, e.g. urine, serum) that is introduced into the helium stream. This results in the formation of hydrated clusters ions, $\text{H}_3\text{O}^+(\text{H}_2\text{O})_3$ at a mass-to-

charge ratio, $m/z = 73$, and their isotopic variant ions H_8DO_4^+ and $\text{H}_9^{17}\text{OO}_3^+$ at $m/z = 74$ and $\text{H}_9^{18}\text{OO}_3^+$ at $m/z = 75$. By adopting the known fractional abundance of ^{18}O in water vapour and accounting for the contribution of the isotopic ions $\text{H}_9^{17}\text{O}^{16}\text{O}_3^+$ to the ion signal at $m/z = 74$, a measurement of the 74/75 ion signal ratio under equilibrium conditions provides the deuterium to hydrogen abundance, D/H, in the water vapour sample ($R_{1\text{vap}}$). The corresponding deuterium abundance in the liquid from which the HDO is evaporating, $R_{1\text{liq}}$, is derived from $R_{1\text{vap}}$ using a correction (a coefficient of 0.935 at a liquid sample temperature of 37 °C) that essentially accounts for the difference in evaporation rates of H_2O and HDO from the liquid to the vapour phase (Smith and Spanel 2001, Spanel and Smith 2001). It is important to note that a conventional mass spectrometric approach to analysing breath water vapour for its deuterium content, which would involve electron ionisation, would be seriously compromised since electron collisional ionisation does not produce a predictable distribution of H_2O^+ and HDO^+ ions. In FA-MS, an equilibrium distribution of the analytical ions is produced, according to the theory of isotope exchange, as indicated above.

7.3.2 Protocol of the injection of $\text{D}_2\text{O}/\text{H}_2\text{O}$ mixtures

An accurately known volume of a $\text{D}_2\text{O}/\text{H}_2\text{O}$ mixture of known composition was injected directly into the pulmonary circulation and to monitor the *immediate* changes in breath HDO, and hence the D/H ratio, using the FA-MS real time analytical method. Assuming this is a closed system, then in principle the breath deuterium content, as measured continuously in sequential breath exhalations, should provide an estimate of the volume of water/blood in the pulmonary circulation.

Nine haemodialysis patients were recruited to the study. Permission for the study was obtained from the Local Ethics Committee and informed consent was obtained from each patient. Total body water estimated by bioimpedance analysis was performed prior to deuterium injection. All patients had central venous catheters fitted to facilitate haemodialysis, which was situated in the right internal jugular vein (RIJ). Specified volumes of the sterile D₂O/H₂O mixture (10%, Cambridge Isotope Laboratories, USA) were injected into the RIJ via the catheter and flushed with saline to eliminate dead space in the catheter. The RIJ drains into the right atrium and then passes into the right ventricle of the heart, which then contracts and delivers the deuterated water into the pulmonary vasculature via the pulmonary artery. We presume that thorough mixing of the injected deuterated water and blood has occurred. The water/blood mixture travels through a successive series of arterioles and eventually arrives at the pulmonary alveoli where release of HDO and H₂O occurs into the lung air. The number density ratio of these molecules in the vapour/gas phase depends on their solution phase ratio and the temperature, which must be close to 37 °C. Then the oxygenated blood is returned in the venous system and eventually drains back into the left atrium via the pulmonary vein. This will then be delivered to the rest of the body tissues by the left ventricle. If thorough mixing of the HDO occurs within the pulmonary loops and is sampled by the lungs, this would result in an alveolar air/vapour phase D/H ratio according to that given by the amount of deuterated water injected and the total volume of water in the pulmonary circulation. (Note- the lung, including the airway bronchi and bronchioles, relies on the blood supplied by the bronchial arteries for oxygen and nutrients and is a part of systemic circulation rather than pulmonary circulation).

7.4 Results

7.4.1 Basic demographics and exploratory measurements

Demographics of all HD patients are summarized in Table 7.1. First of all, the amount and concentration of deuterated water needed to be determined that can be injected safely that would result in measurable D/H ratios in exhaled breath following the general principle described before. By estimating the volume of the pulmonary blood and extra vascular water to be a maximum of one litre (1L) (Brigham, Ramsey et al. 1971, Lange and Schuster 1999), the injection of 1 mL of D₂O should result in an increase of the vapour headspace D/H ratio by at least 1000 parts-per-million, ppm, which can be easily handled by the FA-MS analytical technique.

The exhaled breath of the first patient, P1, was first analysed (in several single exhalations) to obtain the baseline value of D/H ratio (which should be close to that of the local tap water which is 150 ppm) and then 10 mL of a 10% mixture of D₂O in pure water was injected into the central venous catheter in the patient's RIJ vein and breath samples were analysed in real time every 30 seconds or so. Breath measurements on a healthy volunteer were interspersed with the patient data as control measure and to check the proper operation of the FA-MS instrument; the invariant D/H value at close to 150 ppm served as verification. The results are presented graphically in Figure 7.3. The D/H ratio increased rapidly after a delay for about 2 minutes and then reached a plateau with the increment in D/H being only about 70 ppm. This was barely 10% of the predicted 1 L of total lung volume on the assumption that the vapour released at the alveolar interface and the D/H ratio in the lung air was undisturbed as it flows along the bronchial tree and left the respiratory system via the mouth for direct sampling.

Further on in the experiment the D/H value began to fall from the plateau value 2 to 3 minutes after injection. Several further sequential injections were performed in the same patient with similar results. The protocol was repeated on another patient, P2, and the results obtained were shown in Figure 7.3, where a similar form of D/H plot is evident.

Table 7.1 Patients demography, TBW and TLW estimation

Patients	Age	Gender	Height (cm)	Weight (kg)	TBW BIA (L)	TBW D_Breath (L)	TBW D_Blood (L)	TLW(L)
1	32	F	164	80.1	32.5	17.99	22.81	NA
2	61	M	161.4	74.6	39.5	24.75	24.51	NA
3	63	M	171.1	88.4	44.1	41.67	44.94	15.5
4	57	F	157.8	66.4	34.6	33.24	35.71	13.9
5	41	M	174.7	76.5	41.5	42.55	34.48	11
6	70	F	167.8	73	37.5	29.82	33.15	18.4
7	79	F	162.6	57.2	33.6	28.99	32	18.5
8	63	M	175.2	88.4	51.1	50.63	48.78	27.5
9	70	M	171.2	71.6	44.4	37.04	43.01	19.4

TBW = total body water, BIA = Bioimpedance analysis, D = Deuterium,

TLW = total lung water, NA=Not applicable.

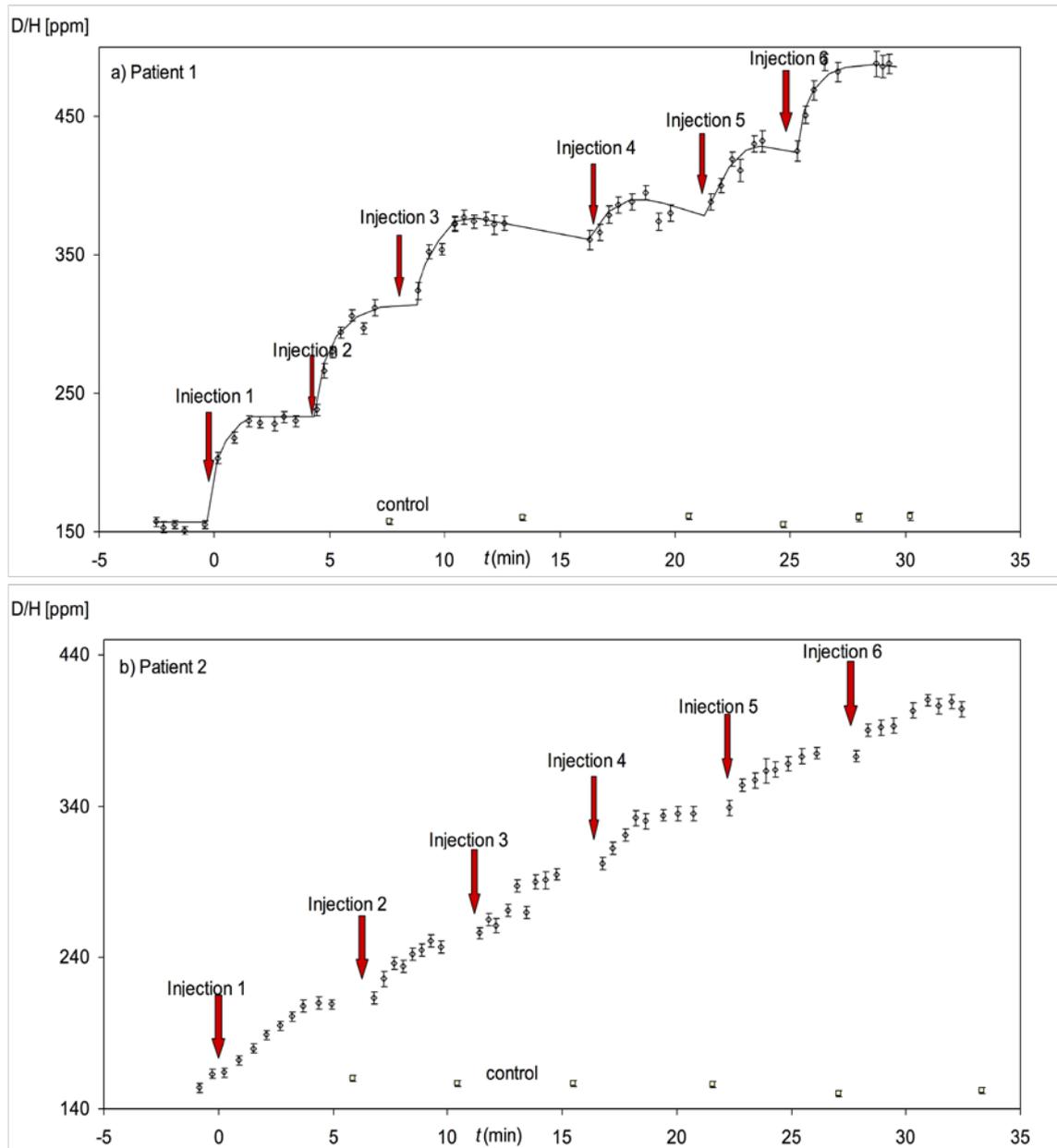


Figure 7.3 Plots against time, t in minutes, of the values of the D/H ratio as measured by FA-MS in parts-per-million, ppm, in single breath exhalations following sequential injections (at times indicated by the arrows) of 10 mL of a sterile 10% mixture of D_2O in pure water into the right internal jugular vein of two haemodialysis patients. Note the pre-injection value of D/H measured as 150 ppm.

7.4.2 Further IV D₂O injections with larger volume

The following patient received a single larger volume (30 ml) of the 10% mixture intravenously and the expected increase and decrease in D/H and the approach towards an equilibrium value from which TBW can be estimated was observed, as exemplified by the oral ingestion data shown in Figure 7.1. After this it was decided that the remaining six patients should received 40 ml of the 10% mixture to further enhance the measured D/H values. The data obtained for all patients showed similar forms after the injection of the 40 ml mixture, and three examples are presented in Figure 7.4. The D/H ratio in the exhaled breath increased rapidly to peak values after about three or four minutes but the fractional increase of the D/H at the peak is still only about 10% of the value deduced from the 1 L estimate of the total lung water volume. The expected decrease in D/H occurs as the deuterated blood enters the systemic circulation as deuterium exchanges with the TBW, similar to that seen in the data in Figure 7.1.

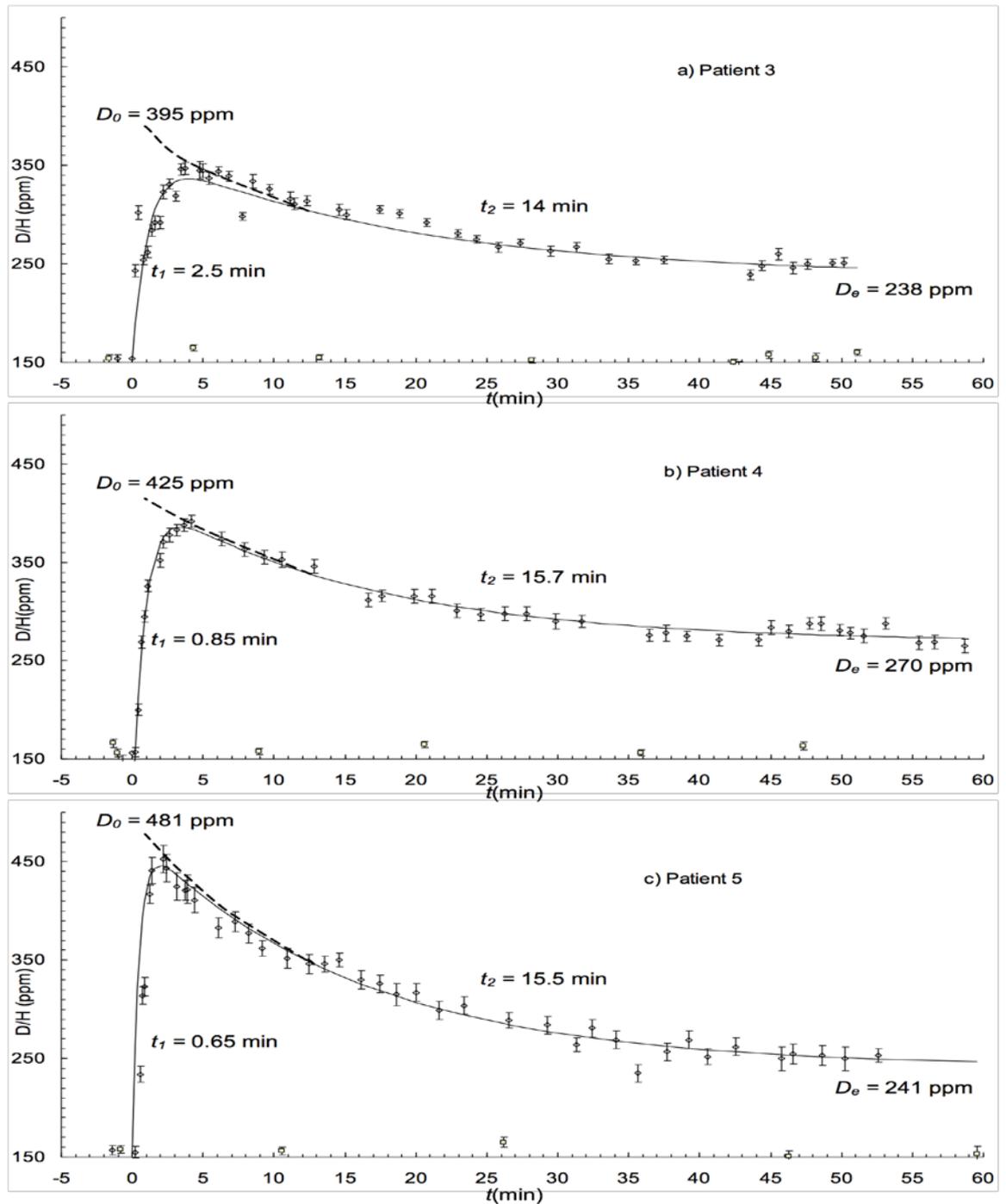


Figure 7.4 Plots against time, t in minutes, of the values of the D/H ratio in parts-per-million, ppm, as measured by FA-MS in single breath exhalations following single injections of 40 mL of a sterile 10% mixture of D_2O in pure water into the right internal jugular vein of three haemodialysis patients, P3, P4 and P5, given in Figures 7.3a, b and c respectively.

It is instructive to fit curves to the data plots given in Figure 7.4, since this can provide four interesting parameters: **(i)** the time constant at early times, t_1 , associated with the increase in the D/H value; **(ii)** the time constant t_2 for the decay of D/H at later times; **(iii)** the late time equilibrium value of D/H, D_e , from which TBW can be estimated; **(iv)** the nominal maximum value for D/H at zero (injection) time, D_0 , that accounts for dispersal of the HDO from the combined effect of pulmonary circulation leaking into the systemic circulation and loss of HDO as the breath passes along the bronchial tree. The functional form describing the time dependence of D/H, indicated as $D(t)$, using the above parameters together with the natural D/H abundance present in breath prior to injection (D_n , typically 150 ppm) may be written as:

$$D(t) = D_e + (D_n - D_0) e^{-t/t_1} + (D_0 - D_e) e^{-t/t_2} \quad (1)$$

The experimental data were fitted numerically by this function using the ordinary least square method of non-linear regression. The results are indicated by the solid lines in Figure 7.4. Additionally, the values of D_0 indicated are obtained by extrapolation using the first and the third terms of the right side of equation (1) only (dashed lines).

The respective parameters can be considered in turn and their likely interpretation.

(i). The time constants t_1 obtained from the data given in Figure 7.4 are: P3, 2.5 min; P4, 0.85 min, P5, 0.65 min. If the pulmonary circulation is completely contained for a period long enough for complete mixing of the HDO in the blood/water volume and no loss of

HDO along the bronchial tree occurred, then the D/H in the exhaled breath would rise rapidly to much higher values than the observed peak values, as explained previously. The speed of the blood flow in the pulmonary circulation is fast at an estimated 1 m/s (calculated from the typical values cardiac output and internal diameters of the jugular vein). Therefore, it is reasonable to conclude that either mixing of the bolus of the injected deuterated water with the pulmonary blood is not rapid and does not evenly distribute into alveolar blood or leakage of this blood into the systemic circulation rapidly occurs or loss of HDO occurs along the bronchial tree. It is likely that all three possibilities occur. What is interesting is that the t_1 for P3 is 3 to 4 times longer than for P4 and P5.

(ii). The time constants t_2 obtained from the data given in Figure 7.4 are: P3, 14.0 min; P4, 15.7 min, P5, 15.5 min. Hence, the approach to equilibrium of the breath D/H value, and hence mixing of D within the TBW, occurs in similar times for all three patients, although it is somewhat faster for P3. The actual values of t_2 in minutes are unremarkable and very similar to those we have commonly observed in healthy volunteers and patients.

(iii). The D_e values obtained from the data given in Figure 7.4 are: P3, 238 ppm; P4, 270 ppm, P5, 241 ppm. To estimate TBW the initial (baseline) value, D_n , of 150 ppm must be subtracted from these figures to provide the increase in D/H, i.e. ΔD , due to the injection of the 4 mL of D₂O. Thus, the ΔD values in ppm are 88, 120 and 91 respectively. The TBW is given by the volume of D₂O (mL) injected divided by ΔD (Davies, Spanel et al. 2001), which gives for P3 45.5 L, for P4 33.3 L and for P5 44.0 L. A more instructive parameter is the percentage water content, %W, of the body as given by (TBW/weight, W). Thus, for P3 (Male; W = 88.3 kg; BMI = 30.2), %W is 51.5 %. For P4 (Female; W = 66.4 kg; BMI =

26.7), %W is 50.2% and for P5 (Male; W= 76.5 kg; BMI = 25.0), %W is 57.5%. These derived %W values are within the expected range (Davies, Spanel et al. 2001, Smith, Engel et al. 2002), but the low value for the male patient P3 is somewhat lower than the average and this is in accordance with his larger BMI, which is indicative of excessive body fat. The lower value of %W for the female P4 is in accordance with expectations that female subjects, on average, have more body fat than males and thus fractionally less muscle in which the body water is largely located. P5 has the ideal BMI and this goes with the mid-range %W for males.

(iv). The D_0 values obtained from the data given in Figure 7.4 are: P3, 395 ppm; P4, 425 ppm, P5, 481 ppm. Following the arguments given above, these would correspond to pulmonary water volumes of about 16, 14.5 and 12.5 L, which are clearly too large (Michard 2007). This anomalous result is likely due to the passage of HDO into the systemic circulation and loss along the airways that reduces the breath HDO concentration. Therefore original assumptions do not hold and the revised flow diagram of HDO/water mixture pathway is demonstrated in Figure 7.5.

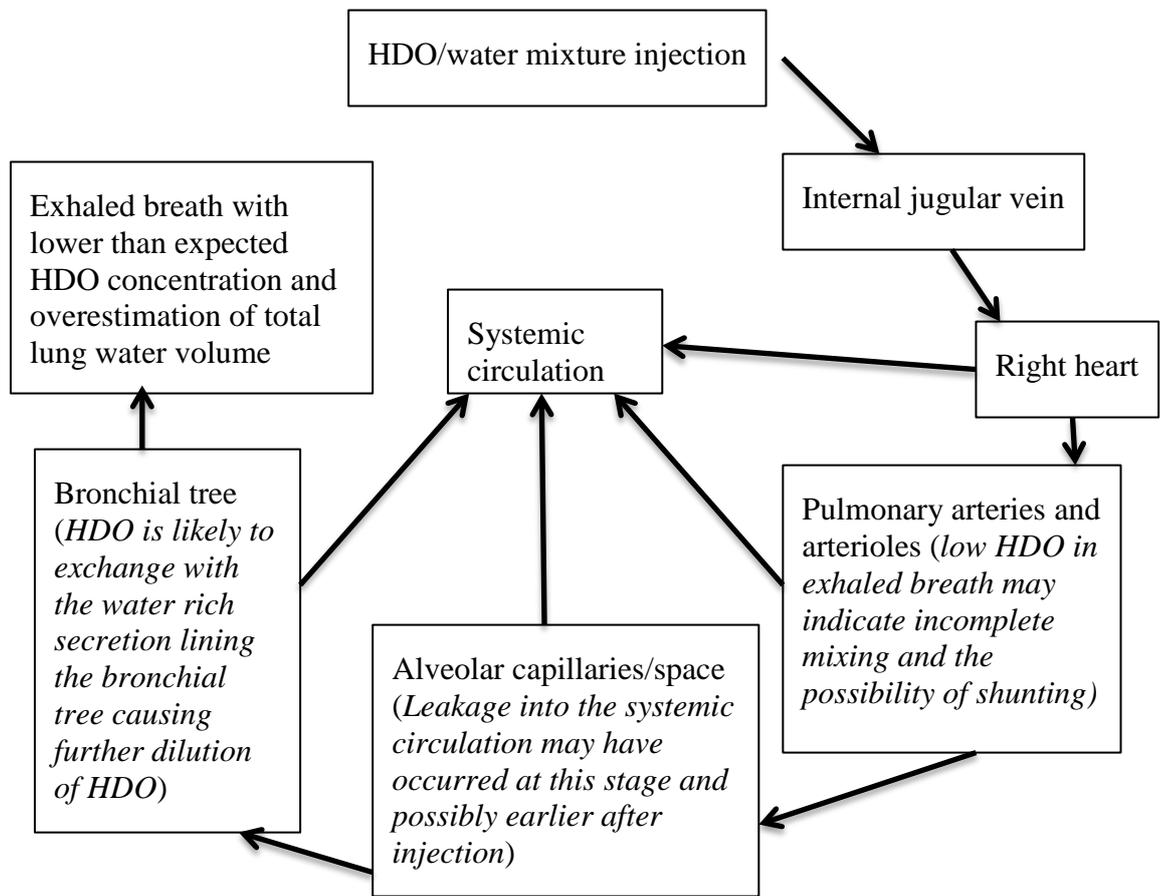


Figure 7.5 Revised flow diagram showing the pathway of HDO/water mixture following injection into the internal jugular vein. The low HDO concentration in the exhaled breath is incompatible with the original assumptions (see Figure 7.2).

7.5 Discussion

The initial objective of these studies was to research a method for estimating lung water by breath analysis, which, if successful, would be of great value to clinicians. But it was immediately obvious that the chosen approach was not viable and so the goal of lung water determination via breath analysis is proving to be elusive. However, the experiments have revealed some interesting physiological results that do impinge on the general area of breath research. As outlined before, the loss along the bronchial tree of trace compounds that are released from the blood stream at the alveolar interface is an important phenomenon that must be considered when relating metabolites in exhaled breath to their systemic (blood stream) concentrations. The importance of the influence of the airways is indicated by the loss of HDO in the airways after its deliberate elevation in the pulmonary/alveolar blood stream. This phenomenon is most significant in the early phase after injection, that is, before equilibrium of HDO within the TBW has been reached. Then, D-H exchange between the vapour HDO in the exhaled breath and the H₂O in the water in the airways surfaces can occur, thus modifying the exhaled HDO concentration. However, this does not invalidate the measurements of TBW using the isotope dilution principle because it is the equilibrium breath and blood concentrations of HDO that is utilized for TBW measurement when HDO in both exhaled breath and the water in the airways is equilibrated and exchange losses along the bronchial tree do not occur. This is further supported by the close correlation between TBW by deuterium analysis derived from exhaled breath and blood headspace in this experiment (see Figure 7.6).

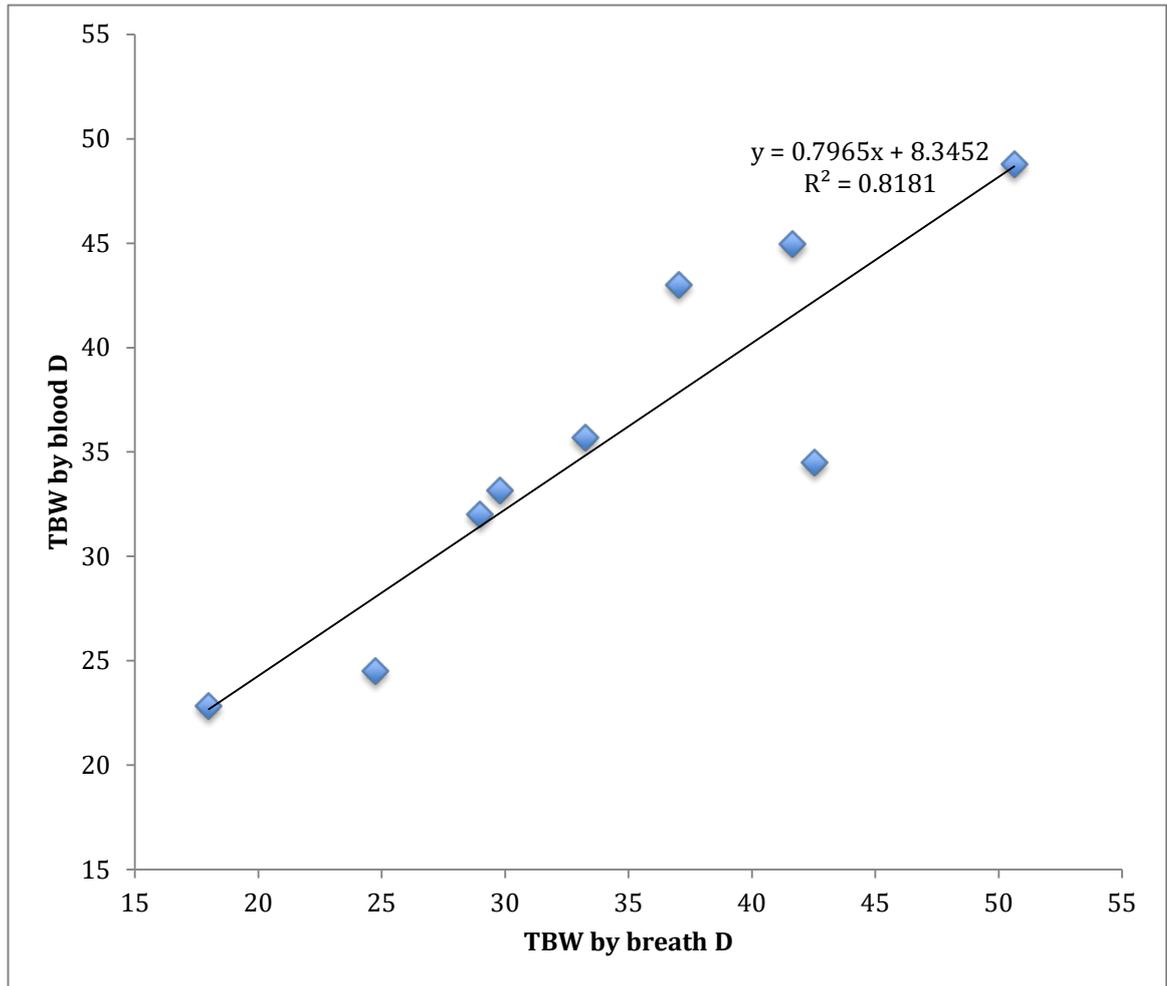


Figure 7.6 TBW estimated by FA-MS deuterium analysis of exhaled breath and blood headspace after equilibration

The significance of the present study to breath analysis in general is clear. If the concentrations of gases released at the alveolar interface are higher or lower than those within the body fluids then the breath concentrations can be increased or decreased as it passes along the bronchial tree. This will be particularly severe for highly water-soluble compounds that are not in systemic equilibrium. Acetone, although highly soluble in water, is also truly systemic being equilibrated amongst the blood, urine, TBW and exhaled breath, and so this compound is undisturbed in breath by passing along the airways. Isoprene, on the other hand, is not very water soluble and not seriously absorbed along the

airways. However, it is soluble in lipids and muscle, which act as a storage compartment for this compound, but exercise releases the isoprene into the blood stream (Koc, King et al. 2011). This release is readily observed by its increase in exhaled breath during the early phase of exercise; it is seen to fall during continued exercise as the source of it diminishes. These influences can presumably occur for other metabolites, so the temptation to tacitly relate breath concentrations to blood concentrations must be done with circumspection for each individual trace compound.

7.6 Conclusions

In conclusion, the measurement of HDO in exhaled breath and the maximum D/H abundance following the injection of deuterated water into the pulmonary and alveolar circulation via the dialysis catheter in haemodialysis patients was very much lower than anticipated. This has vastly overestimated the predicted volume of the total lung water and therefore rendering the accurate measurement of this important clinical parameter to detect and monitor pulmonary oedema using this technique unfeasible. We have proposed a number of possible reasons for the reduced D/H in the exhaled breath. The data presented in study indicate that the concentrations of soluble compounds in exhaled breath do not always correspond to their equilibrated alveolar concentrations. Whilst this is now clear to some researchers, as well explained in the literature (Anderson, Babb et al. 2003, King, Unterkofler et al. 2011, Koc, King et al. 2011, King, Unterkofler et al. 2012), the assumption of equivalence between exhaled and alveolar breath still appear in the current literature. This study strives to contribute to the clarification of this issue.

Chapter 8 Conclusions and future directions

8 Chapter 8 Conclusions and future directions

One of the primary objectives of renal replacement therapy is to attempt to restore euvolaemia by a combination of ultrafiltration by dialysis, fluid restriction and dietary modification, in particularly curbing salt intake. There is a growing evidence to demonstrate that volume expansion is common among dialysis patients and more importantly, its association with markers of cardiac dysfunction such as raised cardiac biomarkers, left ventricular hypertrophy, inflammation and muscle wasting, all of which predict mortality. Cardiovascular disease (CVD) represents the leading cause of death in dialysis patients, responsible for 40 to 60% of all deaths in the United States and Australasia (Johnson, Dent et al. 2009) and some studies also suggest PD patients suffer from higher risk of congestive heart failure and myocardial infarction compared with the HD patients (Johnson, Dent et al. 2009, Kim, Kim et al. 2015).

LVH is an important predictor of cardiovascular death (Parfrey, Harnett et al. 1990, Foley, Parfrey et al. 1995) and PD patients have been found to have higher incidence of LVH and overhydration compared to HD patients in several prospective studies (Takeda, Nakamoto et al. 1998, Enia, Mallamaci et al. 2001). The progressive loss of RRF and altered membrane transport status causing UF failure with time on PD treatment could have a negative impact on hydration status. On the other hand haemodialysis patients are not subjected to such constraints and the fluid status can be manipulated readily by altering the desired UF volume. A retrospective study suggested that long term good BP control and regression of LVH could be achieved with tight control of fluid status by means of progressive UF and strict salt restriction (Ozkahya, Ok et al. 1998). Similar results were demonstrated in a prospective randomised trial using BIA target fluid removal in HD

patients (Hur, Usta et al. 2013). Onofriescu et al showed survival benefit in HD patients where strict volume control was achieved by BIA guided UF although cardiac assessments by echocardiography were not performed (Onofriescu, Hogas et al. 2014).

The conventional method of fluid assessment based on physical examination is subjective and crude. Clinical biometrics such as blood pressure and body weight are informative but often confounded by the presence of cardiac dysfunction, multimorbidity and imperceptible changes in body composition longitudinally without any significant deviation from baseline weight. The practice of titrating target dry weight down to achieve normovolaemia without a validated clinical monitoring system risks hypovolaemia, causing loss of residual renal function in PD and cardiac stunning in HD patients, both associated with poor survival (Bargman, Thorpe et al. 2001, Termorshuizen, Dekker et al. 2004, Selby and McIntyre 2007). Accurate assessment and monitor of fluid status to inform clinical decision is therefore a crucial part of renal replacement therapy.

One of the potential candidates to aid clinical assessment of fluid status is bioimpedance analysis. The derived impedance when an alternating current is applied to biological tissue at a given voltage has two components: resistance which is inversely proportional to the total content (intracellular and extracellular water) and reactance, a measure of electrical capacitance which is proportional to the cell mass. The two parameters can be used in the vector format called bioimpedance vector analysis, after adjusted for height, to track changes in body composition longitudinally (Piccoli 1998). These simple measurements can be further transformed into volumetric expression of different fluid compartments such as ECW and TBW, often with assumptions incorporated into the algorithms.

Overhydration as determined by vectors or ECW/TBW ratio has been shown to correlate with worse survival in dialysis cohorts (Pillon, Piccoli et al. 2004, Paniagua, Ventura et al. 2010, Koh, Wong et al. 2011).

One of the abnormal features of body composition as determined by BIA in chronic dialysis patients is progressive reduction in muscle mass coupled with disproportional increase in ECW with time, something not dissimilar to other chronic diseases (Plum, Schoenicke et al. 2001, Woodrow, Oldroyd et al. 2004, van Biesen, Claes et al. 2013). Overhydrated patients tend to be older, more likely to be diabetic, have more comorbidities and have a lower serum albumin. Hypoalbuminaemia has been shown to predict poor survival in patients on dialysis and it is much more prevalent in PD than HD patients, partly due to significant peritoneal protein loss but increasingly seen to be associated with inflammation (Kaysen, Yeun et al. 1997, Plum, Schoenicke et al. 2001). In patients on PD, hypoalbuminaemia is strongly associated with overhydration as determined by BIA but it was not clear if the excess fluid is distributed equally between the intravascular and extravascular compartment.

The study in chapter 3 confirmed the association between raised ECW/TBW ratio and low albumin and inflammation in PD patients and the measured fluid excess was predominantly in the interstitial space, *i.e.* extravascular, given that the measured plasma volume was not expanded. In the multivariate analysis, hypoalbuminaemia became the only predictor of tissue overhydration, displacing other relevant clinical determinants of hypervolaemia. We also proposed a different approach to characterising tissue overhydration by using the difference between measured TBW by deuterium dilution and

estimated by BIA which correlated with the difference between the measured and estimated ECW/TBW by BIA. The advantage of this approach is that it combines an absolute determination of body water with a technique that relies on data derived from populations that often differ from the relatively sick dialysis population to which it is being applied. This is especially the case at the extremes of body composition such as obesity and malnutrition. This approach also demonstrated PD patients to be overhydrated, especially when hypoalbuminaemic. The clinical implications of this are twofold:

(a) given the high frequency of hypoalbuminaemia in PD patients due to their peritoneal protein losses (transport status being a function of intraperitoneal inflammation and the single most important determinant of plasma albumin concentration), then inevitably tissue overhydration will be more common in PD than HD patients, and

(b) that any attempt to normalise fluid status in the context of hypoalbuminaemia may unnecessarily cause central volume depletion and loss of RRF. No intervention currently exists that can alter this, but it is reassuring that the reduced survival associated with lower plasma albumin, seen in both dialysis modalities, is if anything less marked in PD patients (Mehrotra, Duong et al. 2011).

The cardiac sub-study in chapter 3 revealed that a high proportion of PD patients (93%) had NYHA class II and III heart failure symptoms with varying degree of LVH (62%). The predominant pathology was diastolic dysfunction and many fulfilled the diagnostic criteria of HFNEF. Biomarker of cardiac damage such as serum BNP and elevated ECW/TBW ratio determined by BIA have been shown to predict survival in PD (Paniagua, Amato et

al. 2008, Koh, Wong et al. 2011) but the relationship between BNP and differing fluid compartments remains unclear. We found for the first time in this study, no correlation between BNP and intravascular volume as measured by plasma volume, or other validated echocardiographic parameters of left ventricular filling pressure such as E/E' or E/A. Although the measured plasma volume is in the normal range in over 85% of the patients, PD patients with HFNEF tended to have higher plasma volume. We propose that higher intravascular volume may be needed for adequate ventricular filling to compensate for reduced myocardial compliance but the real clinical significance is unclear at present. This is further reason to be cautious about normalising fluid status in this patient population until appropriate studies have been conducted.

BIA is a highly reproducible measurement, with inter-observer and intra-observer error of less than 2%. It is an ideal technique to monitor and measure impact of intervention on changes in body composition, as shown in the longitudinal study of icodextrin by Davies et al (Davies, Woodrow et al. 2003). We hypothesized that PD patients develop worsening of fluid status over time on treatment due to loss of RRF and progressive muscle wasting and BIA has the potential to aid clinical decision by detecting and informing such changes. This hypothesis was tested in chapter 4 where we undertook a prospective, randomised, open-label, blinded end point controlled trial in 3 UK centres (Stoke, Sheffield and Leeds) and 1 Chinese centre (Shanghai) to evaluate the clinical benefit of having longitudinal bioimpedance measures presented as vector plots to help clinicians maintain stable fluid status over 12 months. The primary endpoints were changes in ECW, TBW volumes and (ECW/TBW ratio) by converting the resistance and reactance data using algorithm based on the software provided by the BIA device after study completion. The decision to use bioimpedance to monitor the direction of body composition change using vector plots

rather than attempting to normalize fluid status from predicted normal volumes was based on safety concern, specifically to avoid risk of hypovolaemia causing premature loss of RRF, largely based on our observations in chapter 3. We found overall stability over the study period of bioimpedance derived fluid volume non-anuric patients, whereas, as predicted, fluid status worsened due to loss in total body water, indicative of muscle wasting, without a concomitant fall in the ECW. This effect was ameliorated by use of BI in the Shanghai population, but the effect seems to have been achieved by increasing the glucose prescription and preventing loss in TBW, so the mechanisms of action may be different to that initially postulated. Nutritional status is a key determinant of body composition, so the additional calories supplied by the increased glucose prescription could have been the explanation. The aim to optimise fluid status by setting a lower target weight in the UK non-anuric bioimpedance intervention group may have had the opposite effect as it led to the reduction in TBW without any parallel change in ECW or ECW/TBW ratio. Marginal benefit was seen in the bioimpedance intervention group in Shanghai anuric patients where fluid status was better maintained compared to the control group. Taken as a whole, the impact of BIVA on informing clinical decision for fluid management in PD patients was small, not least because of the stability of fluid status in non-anuric patients and certainly more complex than anticipated.

For example, one interesting aspect of the study was how the availability of bioimpedance information affect clinical decision making, given patient's target weight was more likely to be reduced compared to the control group where changes in target weight were very balanced. This could be due to study bias or clinicians may be more inclined to reduce target weight in response to another clinical parameters, such as hypertension. Little is known about how patients have actually responded to requests to reduce target weight.

Suboptimal adherence to dialysis treatment, dialysis prescription and dietary advice is common in dialysis population which leads to poor clinical outcome and there are evidence to suggest psychological interventions may improve treatment compliance (Clark, Farrington et al. 2014). This interesting but complex area deserves further evaluation in future study.

Another signal coming from this study is the importance of maintaining residual kidney function. In the cardiac sub-group analysis of the longitudinal fluid management by BIVA described in chapter 5, all patients in this single centre study showed progressive worsening of systolic and diastolic functions characterised by reduction in LVEF and increased in E/E' over the 12 month period but patients in the BIVA group had better preservation of cardiac function and RRF. Multivariate analysis indicated that the most important predictors of the echocardiographic parameters at 12 months were the respective baseline cardiac measurements, comorbidity and randomisation group. It may be tempting to assume the incorporation of BIVA in fluid management helps to preserve cardiac function longitudinally but it is a premature suggestion and potential confounders need to be considered. The degree of cardiac preservation may be statistically significant but the clinical and functional benefits are not clear. The BIVA group had more patients compared to the control group as a result of unbalanced randomisation process and they were older with worse E/E' ratio at baseline. Therefore it is possible that the absolute deterioration in cardiac function was not so great simply because of the poorer baseline measurement. However the relative preservation of RRF is the best candidate for a mechanism given previous evidence that this is strongly associated with cardiac deterioration (Wang and Lai 2006), providing more evidence if it were needed of the value of this commodity to dialysis patients. Further studies using BIA should consider using this as their surrogate

endpoint, especially following the recently performed meta-analysis of interventions on left ventricular hypertrophy showing the lack of translation into clinically relevant outcomes, specifically survival (Badve, Palmer et al. in press 2016).

The last two chapters of the thesis explored the potential of breath analysis of deuterium abundance by FA-MS as a novel technique to measure lung water volume. Pulmonary oedema is a potential life threatening condition characterised by excessive accumulation of fluid in the alveolar space causing impaired gaseous exchange and hypoxaemia. Asymptomatic or subclinical pulmonary oedema was confirmed in haemodialysis patients by indicators dilution method and removed by dialysis treatment (Wallin, Jacobson et al. 1996). Current analytical techniques to evaluate lung water volume are invasive and results are often delayed due to complicated sample processing, seriously compromise its use in a busy outpatient dialysis unit. FA-MS offers a potential solution to this problem with its on-line, real time measurements to inform clinical decision and more importantly, it is patient-friendly. Deuterium abundance in exhaled breath was measured using FA-MS after mouth inhalation in the first experiment, followed by intravenous injection of the known quantity of deuterium mixture in the second. However, the deuterium measurement in the breath was far lower than expected and this vastly overestimated the volume of distribution of lung water. It is therefore not a feasible tool to estimate lung water in its current form. This has raised questions regarding the assumption that the concentrations of soluble compounds are equivalent both in exhaled breath and the equilibrated alveolar space by failing to take into account the 'dilutional' phenomenon as HDO exchange with water molecules in the mucosal lining of the bronchial tree which can be substantial.

Final reflections

1. Pre-existing pathologies such as heart failure, hypoalbuminaemia, comorbidities are more important determinants of abnormal fluid status than dialysis therapy and the associated ultrafiltration limitations. There is currently a lack of effective interventions to optimize fluid status.

2. There are limitations in the current techniques to measure and monitor fluid status. Although BIVA is easy to use and patient friendly, the significance of vector changes longitudinally is difficult to understand without important data regarding lean muscle and fat. The graphical representation of changing fluid status poses a clinical challenge for clinicians who are more familiar with absolute volume expression. Large scale plasma volume measurement is limited by the inherent radioactivity and lung water measurement by FAMS is not a feasible tool at present.

3. Residual kidney function is repeatedly shown as being important to fluid management and maintaining stable cardiac function, nutritional status and body composition. Preserving this should continue to be a goal for future research.

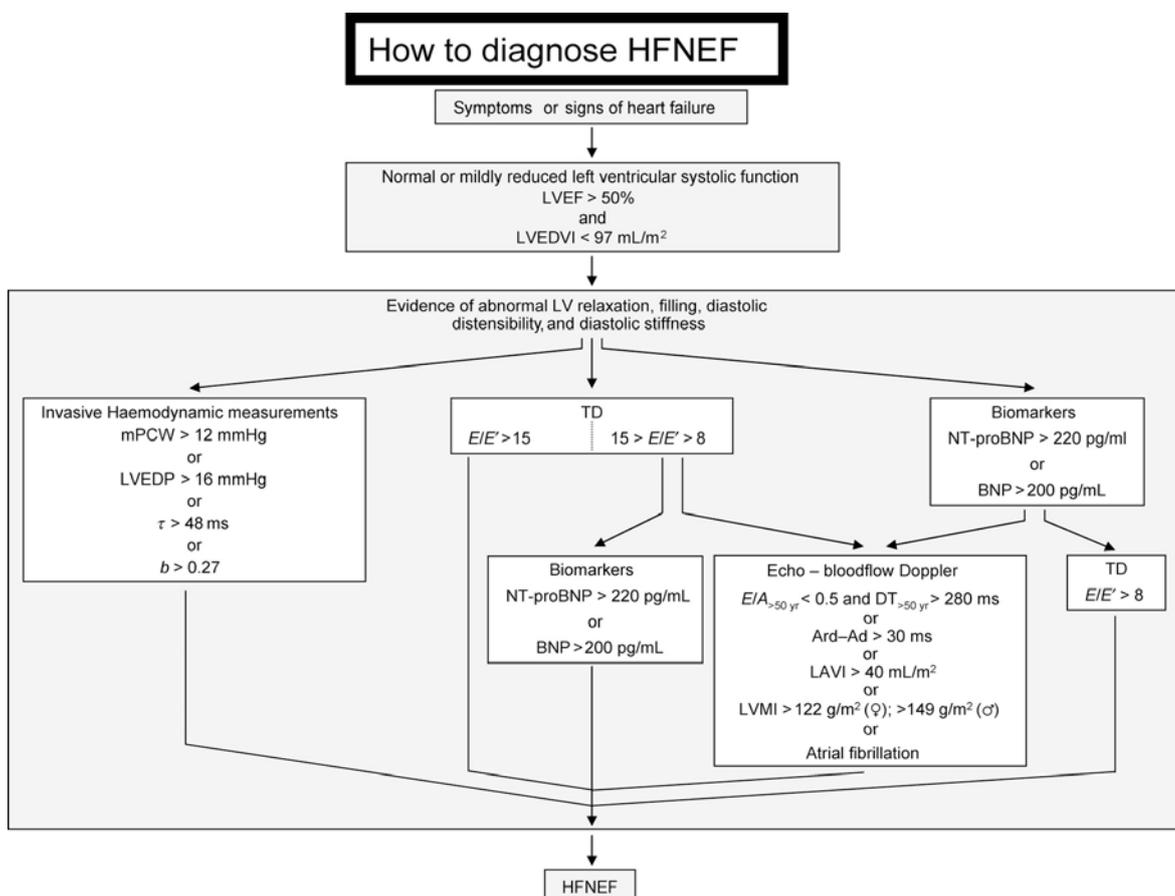
4. Methods of intervention to optimise fluid status are different between dialysis modalities. For PD it is the interplay between altering PD prescription with varying degree of glucose exposure to enhance ultrafiltration and advising patients to adjust target weight likely involves calorie restriction which may not be desirable.

5. There is a need for future qualitative studies to assess patient's understanding and behaviour in response to clinical advice to optimize fluid status.

Appendix

Appendix

Appendix 1: Diagnostic flowchart on ‘How to diagnose HFNEF’ in a patient suspected of HFNEF.



LVEDVI, left ventricular end-diastolic volume index; mPCW, mean pulmonary capillary wedge pressure; LVEDP, left ventricular end-diastolic pressure; τ , time constant of left ventricular relaxation; b , constant of left ventricular chamber stiffness; TD, tissue Doppler; E , early mitral valve flow velocity; E' , early TD lengthening velocity; NT-proBNP, N-terminal-pro brain natriuretic peptide; BNP, brain natriuretic peptide; E/A , ratio of early (E) to late (A) mitral valve flow velocity; DT, deceleration time; LVMI, left ventricular mass index; LAVI, left atrial volume index; Ard, duration of reverse pulmonary vein atrial systole flow; Ad, duration of mitral valve atrial wave flow (Paulus, Tschope et al. 2007).

Appendix 2: The transformation of the resistance and reactance measurements to TBW and ECW using Akern BIA 101 Anniversary device.

172 sets of patient information (including gender, height and body weight) and resistance and reactance were entered into the software of Akern BIA 101 Anniversary. The TBW and ECW output from the software were recorded. Based on the work by De Lorenzo *et al* (De Lorenzo, Andreoli et al. 1997) the first 100 records was used to generate a set of formulas to calculate TBW and ECW from height, body weight, gender and raw resistance and reactance readings. The formulas were then applied to calculate TBW and ECW for all records. The formulas generated were as following.

For TBW, If female,

$$TBW = 0.511 \times \frac{\text{height}^{\frac{4}{3}} \times \text{weight}^{\frac{1}{3}}}{\text{resistance}^{\frac{2}{3}}} + 6.87$$

For TBW, if male

$$TBW = 0.608 \times \frac{\text{height}^{\frac{4}{3}} \times \text{weight}^{\frac{1}{3}}}{\text{resistance}^{\frac{2}{3}}} + 3.37$$

For ECW

$$ECW = \frac{TBW}{1 + 22.907576 \left(\frac{\text{resistance} + \text{reactance}}{\text{reactance}} \right)^{-1.255081}}$$

Appendix 3: Ethical approval for 'Developing BIA as a tool for fluid management in PD patients: A validation study.'


National Research Ethics Service
North Staffordshire Local Research Ethics Committee
North Staffordshire Medical Institute
Hartshill Road
Hartshill
Stoke-on-Trent
Staffs
ST4 7NY
Telephone: 01782 714935

30 March 2009

Professor Simon J Davies
Professor of Nephrology and Dialysis Medicine
University Hospital of North Staffordshire
Princes Road
Hartshill
Stoke-on-Trent, Staffordshire
ST4 7LN

Dear Professor Davies

Full title of study: Developing Bioimpedance (BIA) as a tool for fluid management in Peritoneal Dialysis Patients: A validation study.

REC reference number: 09/H1204/19

Thank you for your letter of 10 March 2009, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Vice-Chair.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Ethical review of research sites

The favourable opinion applies to the research sites listed on the attached form

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission at NHS sites ("R&D approval") should be obtained from the relevant care organisation(s) in accordance with NHS research governance arrangements. Guidance on applying for NHS permission is available in the Integrated Research Application System or at <http://www.rdforum.nhs.uk>.

This Research Ethics Committee is an advisory committee to West Midlands Strategic Health Authority
The National Research Ethics Service (NRES) represents the NRES Directorate within
The National Patient Safety Agency and Research Ethics Committees in England

**North Staffordshire Local Research Ethics Committee
LIST OF SITES WITH A FAVOURABLE ETHICAL OPINION**

For all studies requiring site-specific assessment, this form is issued by the main REC to the Chief Investigator and sponsor with the favourable opinion letter and following subsequent notifications from site assessors. For issue 2 onwards, all sites with a favourable opinion are listed, adding the new sites approved.

REC reference number:	09/H1204/19	Issue number:	1	Date of issue:	30 March 2009
Chief Investigator:	Professor Simon J Davies				
Full title of study:	Developing Bioimpedance (BIA) as a tool for fluid management in Peritoneal Dialysis Patients: A validation study.				
<p><i>This study was given a favourable ethical opinion by North Staffordshire Local Research Ethics Committee on 30 March 2009. The favourable opinion is extended to each of the sites listed below. The research may commence at each NHS site when management approval from the relevant NHS care organisation has been confirmed.</i></p>					
Principal Investigator	Post	Research site	Site assessor	Date of favourable opinion for this site	Notes ⁽¹⁾
Professor Simon J Davies	Professor of Nephrology	University Hospital of North Staffordshire	North Staffordshire Local Research Ethics Committee	30/03/2009	
<p>Approved by the Chair on behalf of the REC: <i>Janet Clarke</i> (Signature of Chair/Co-ordinator) (delete as applicable) JANET CLARKE (Name)</p>					

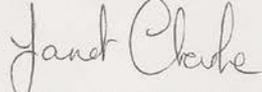
(1) The notes column may be used by the main REC to record the early closure or withdrawal of a site (where notified by the Chief Investigator or sponsor), the suspension of termination of the favourable opinion for an individual site, or any other relevant development. The date should be recorded.

09/H1204/19

Please quote this number on all correspondence

With the Committee's best wishes for the success of this project

Yours sincerely



Dr Mark Gunning pp
Vice Chair

Email: Janet.Clarke@uhns.nhs.uk

Enclosures: "After ethical review – guidance for researchers"
Site approval form

Copy to: Dr D Clements, R&D Manager, UHNS

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

<i>Document</i>	<i>Version</i>	<i>Date</i>
Response to Request for Further Information		10 March 2009
Participant Consent Form	2.0	10 March 2009
Participant Information Sheet	2.0	10 March 2009
Statistician Comments		09 January 2009
Peer Review	from funder	27 November 2008
Covering Letter		12 January 2009
Protocol	1	15 October 2008
Investigator CV		
Application		14 January 2009

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Now that you have completed the application process please visit the National Research Ethics Website > After Review

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

The attached document "After ethical review –guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

We would also like to inform you that we consult regularly with stakeholders to improve our service. If you would like to join our Reference Group please email referencegroup@nres.npsa.nhs.uk.

Appendix 4: Inclusion letter for BIA guided fluid management study on the NIHR CRN Portfolio.



NHS
**National Institute for
Health Research**

NIHR Clinical Research Network
Coordinating Centre
Fairbairn House
71-75 Clarendon Road
Leeds LS2 9PH

Tel: 0113 343 2314
Fax: 0113 343 2300
Email: info@ukcrn.org.uk
www.crmcc.nihr.ac.uk

13th August 2009

Professor Simon J Davies
Institute of Nephrology and Dialysis Medicine
Keele University
Thornburrow Drive
Hartshill
Stoke on Trent
Staffs
ST4 7QB

Dear Professor Davies

Re: Developing Bioimpedance (BIA) as a tool for fluid management in peritoneal dialysis patients : A validation study (IRAS Ref: 10790)

The study detailed above has now proceeded through National Institute for Health Research Coordinated System for Gaining NHS Permission (NIHR CSP) successfully and I am pleased to confirm that it is eligible for inclusion on the National Institute for Health Research Clinical Research Network (NIHR CRN) Portfolio.

Please note that recruitment/accrual study data must be uploaded every month as a condition to be on the NIHR CRN Portfolio. Please be aware that accrual data is monitored and the CLRN is notified if the study is not uploading accrual data.

It is your responsibility to:

- identify and forward (by return post and/or email) the name and contact details of the person who will be responsible for uploading the accrual data for your study. The named person is referred to as the 'accrual contact'
- ensure that the accrual contact uploads recruitment/accrual data regularly on a monthly basis. Reported accrual activity ultimately informs the allocation of funding for NHS support
- confirm whether the study is open to new sites. This information is extremely important to the successful development of studies.

We will then:

- enter the study on the NIHR CRN Portfolio upon the receipt of accrual contact's details
- forward an accrual data package with detailed instructions on how to upload the data to the accrual contact.

Thank you for your support in this process which will be critical to the successful development of NIHR CRN Portfolio. Our aim is to ensure the provision of high

In partnership with



Directors
Professor Peter Selby
Professor Janet Darbyshire

quality infrastructure to support clinical research in the NHS and support the delivery of your study.

Please do not hesitate to contact me should you require further information

Best Wishes



Dr Sam Taylor
Portfolio Lead
NIHR Clinical Research Coordinating Centre (NIHR CRN CC)
Fairbairn House
71-75 Clarendon Road
Leeds
LS2 9PH

Tel: 0113 343 0403
Fax: 0113 343 2300
Email: sam.taylor@nihr.ac.uk
www.crnc.nihr.ac.uk

Appendix 5: Patient information leaflet and consent form for BIA guided fluid management study.

University Hospital of North Staffordshire 
NHS Trust

PATIENT INFORMATION LEAFLET

(Version 2.0 dated 10th March 2009)

Project title: Bioimpedance as a tool for fluid management in PD patients

Invitation to participate

We are asking if you are willing to take part in a clinical study. Please take time to read and consider the following information carefully, and feel free to discuss it with whomever you wish. If anything is unclear, please do not hesitate to contact your consultant or your named nurse.

What is the purpose of the study?

Patients with kidney failure often have abnormal fluid balance, especially when treated with dialysis. When peritoneal dialysis (PD) patients have excess fluid in their body this can cause high blood pressure and heart disease. When too much fluid is removed through dialysis the patients can feel unwell with dehydration and reduced urine production. In order to avoid this we need to have a simple, accurate and patient-friendly clinical tool that is able to monitor fluid status over time and give useful information for the doctors to make sound clinical decision for each individual patient. Unfortunately we do not have such method that has been properly tested in our day-to-day patient care at present.

Bioimpedance (BIA) is a clinical device that has been used extensively in research and some clinical settings to measure body fluid compartment. It is likely that it will become the standard tool to help doctors in assessing fluid status. It is simple to perform and non-invasive. By doing this study, we hope to demonstrate that regular BIA measurement will give doctors added information regarding patient's fluid status so that subsequent clinical therapy can be tailored and applied to restore optimal fluid balance. We also want to investigate if blood pressure and heart function can be improved once we achieve better fluid status.

Why have I been chosen?

You have been chosen because you are treated with peritoneal dialysis.

Do I have to take part?

No. If you are unable to take part for whatever reason (you do not have to give one), then this does not matter. If you do decide not to take part in this study, your treatment shall not be affected in any way.

What will happen to me if I take part?

This study will last 12 months. During this time when you having your routine assessments, home visits or attending clinic you will undergo a BIA test to assess your fluid status. This is a randomized study, which in this case means that the information from the BIA assessment will be available to the doctors to interpret on a random basis. If you are in the control group, decided by chance, the information is collected but not acted upon and your dialysis schedule will not be affected. If you are in the 'active' group, then your doctor may wish to adjust your target dry weight according to the additional information obtained from the BIA.

Can I withdraw from the study at a later date?

Yes. You are free to withdraw from the study at any stage even if you had agreed initially. This will not affect your treatment.

What do I have to do?

Once you have agreed to take part in the study you will be asked to continue your dialysis as usual and you will be assessed in the normal clinical way to decide if your fluid status is satisfactory. If it is you will start the study which last 12 months.

During this time, in addition to your routine assessments you will be asked to have the following done on a 3 monthly basis:

- Assessment of fluid status by bioimpedance. You will have sticky pads on your hand and foot while lying down and connected to a device. A small electric current is passed through the body that you cannot feel and the measurements are used to calculate the amount of fluid present. You will feel no pain during the measurement and it takes about 5 minutes. It is very similar to having an ECG done.
- Measurement of blood pressure.
- Additional blood sample (approximately 10ml) taken along with your routine blood tests for research purpose.

We will try to incorporate the visits into your regular clinical appointment as much as possible to reduce hospital attendance.

Additional tests: We would like you to have these additional tests if you are able

1. There is the option to undergo an echocardiogram to assess your heart function. This involves placing some jelly on your chest and looking at the heart with a probe on a small screen. As part of the test we would like to ask you to perform some very gentle exercise for about 5 minutes on a gym bicycle if you are able to. This is equivalent to walking on a flat surface at a moderate pace for a similar amount of time. This procedure will give important additional information about your heart

function. Your heart will be monitored closely throughout the procedure and if you do not feel well during the test you can stop at any time. The test will last for one hour and transport will be provided if required. If you do not want to participate in this additional test (heart scan) but agree for the rest of the study, it is acceptable.

2. Total body water measurement by deuterium analysis. Deuterium is heavy water which can be found in small quantity in tap water and it is safe and not radioactive. Patients will be asked to drink a small amount of deuterium and the blood tests obtained before and after the ingestion will be used to determine the total amount of water in your body.

What are the side effects or risks of taking part?

We do not anticipate any side effects or risks during this study as BIA measurement is non-invasive. There may be some discomfort when blood is being taken, so where possible we will take this when you are having blood taken for your usual clinical monitoring.

What are the possible benefits of taking part?

The results of this study are likely to help doctors improving their management of future PD patients. You are unlikely to receive any benefit of taking part.

What if new information becomes available?

If any new information comes to light to suggest any harm to you the study will be stopped.

What about traveling costs?

You will be re-imbursed any travelling costs incurred by travelling to the hospital for the purpose of the study.

What happens when the research study stops?

The result of the study will be analysed to see if BIA measurement is a useful clinical tool to monitor fluid status in PD patients. This would help doctors to refine their clinical care of the patients and facilitate design of future studies.

What happens to the results of the research study?

It will be presented at scientific meetings and published in international research journals.

Who is organising the research and funding?

This is a multi-centre study involving renal units in North Staffordshire, Leeds and Sheffield. It is designed and organized by your doctors, led by Professor Simon Davies who is based in North Staffordshire. The study will be funded from research grants

including from a pharmaceutical company that makes dialysis solutions. Your consultant will not be paid for doing this study.

Will my taking part in this study be kept confidential?

Yes. All data collected from this study will be kept anonymous (all patients will be given numbers and cannot be identified from this except by members of the research team). If you consent to take part in the research your medical records may be reviewed for the purpose of analysing results. They may also be looked at by the regulatory authority to ensure that the study is being carried out properly. Your name however will not be disclosed outside your own hospital.

All the information identifying patients will be stored in the hospital in locked rooms if in paper form. Any data stored on computers will be protected by passwords known only to those directly involved in the research project.

Who has reviewed the study?

This study has been reviewed by experts in dialysis treatment who are independent of the study and has been approved by the local ethics committees.

Contact for further information

If you need independent advice regarding participating in research you can contact the PALS office of University Hospital of North Staffordshire on 01782 552814 or INVOLVE organization on 02380651088. This is a national advisory group that support and promote active public involvement in healthcare research. If at any stage you require more information regarding this particular study do contact your own named nurse. Alternatively you can contact the consultant supervising your care, Professor Simon Davies, either via the secretary on 01782 554164 or again via your PD nurse.

PATIENT CONSENT FORM

Bioimpedance as a tool for fluid management in PD patients

I have read the Patient Information Sheet (Version 2.0 dated 10th March 2009) and have had the opportunity to discuss any concerns.

I agree to taking part in this study and that my participation is voluntary and that I am free to withdraw from the study at any time without my treatment being affected.

I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from the research teams, from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.

I understand that I will not benefit financially if this research leads to the development of a new treatment or medical test.

I agree to undergo the heart scans (Echocardiograms) at the hospital.

Patient Name (PRINT) _____
Signature _____

Witness Name (PRINT) _____
Signature _____

Date: ___/___/___

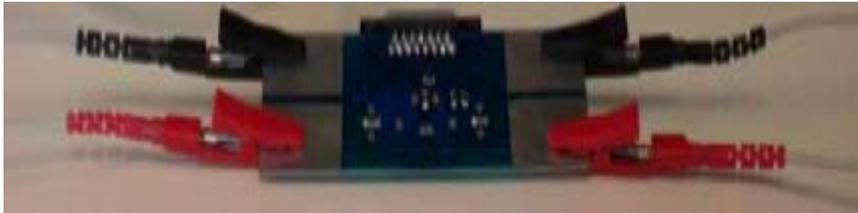
(Version 2.0 dated 10th March 2009)

Appendix 6: BIA 101 Analyser user manual.

IMPORTANT: All measurements must be performed on non-conductive surface

BIA TESTING PROCEDURE

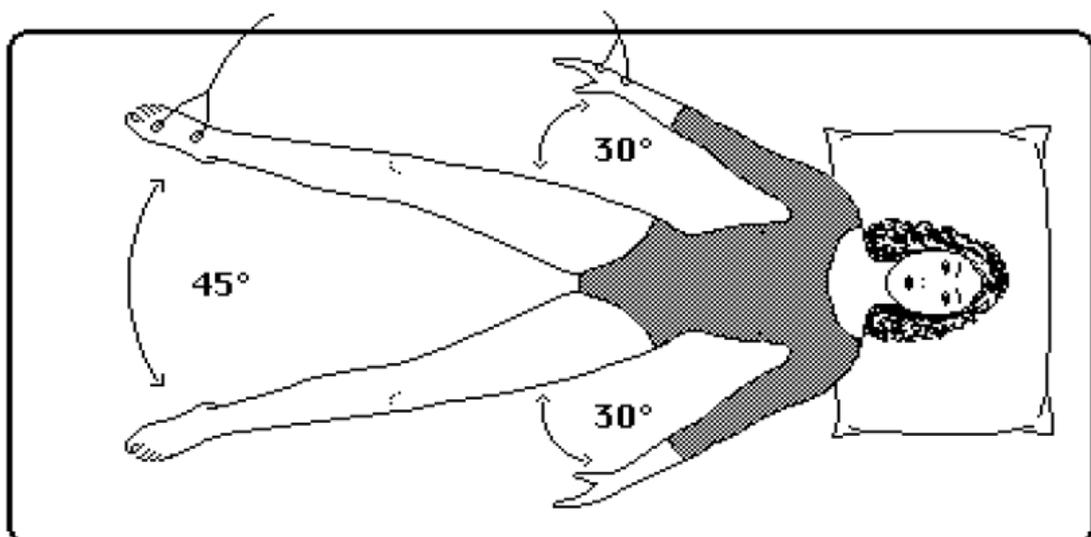
- BIA 101 analyser should be calibrated using the dummy circuit provided. This should be performed at the beginning of each session of patient measurements.



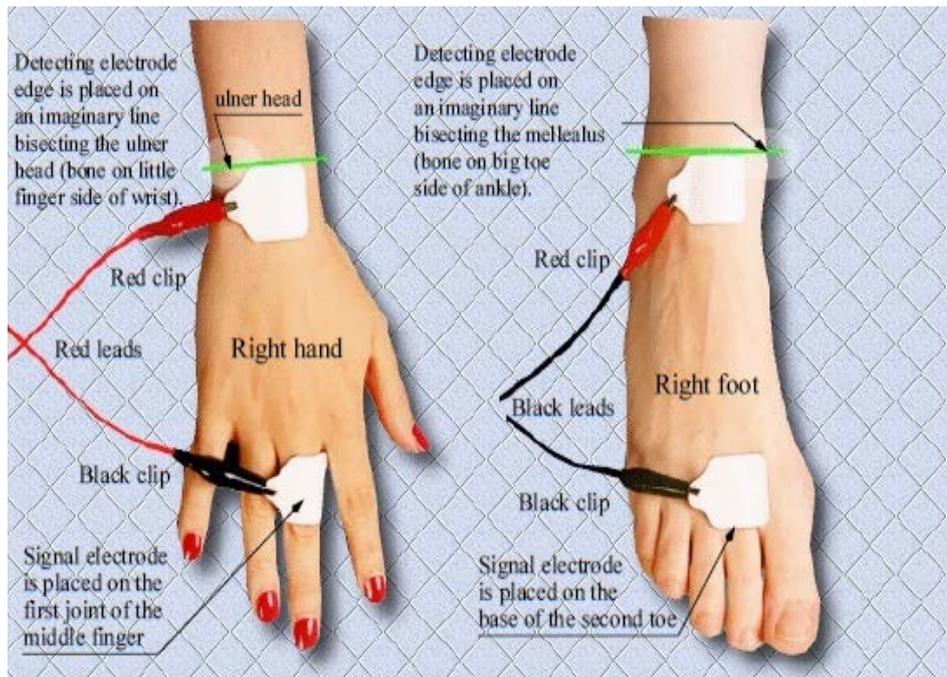
- The reference values of resistance and reactance are printed at the back of the circuit. Measurements within 1% precision of resistance value and 3% precision of reactance value are considered acceptable. All measurements should be recorded in record sheet provided.
- Any abnormal result should be reported back to the study coordinator.

RESEARCH PARTICIPANTS TESTING PROCEDURE

- The participants should remove their right shoe and sock (generally the study is completed on the right side of the body). Whichever side is used should always be used subsequently.
- The participants should lie supine with their arms 30 degrees from their body and legs not touching for about 5 minutes. Jewellery on the electrode side should be removed if possible.



- Attach the electrodes and cables as shown in the illustration.



- Turn the analyzer on and make sure the participant refrains from moving.
- When the measurements have stabilized, record the displayed Resistance (R) and Reactance (Xc).
- Remove and dispose of the electrodes so as to not injure the participant's skin.

Maintenance

- The analyser should be kept in the case provided when not in use.
- It is recommended to charge the batteries fully at least once a week. This usually takes approximately 12 hours.

Appendix 7: Deuterium Oxide quality assurance report.

Product: Deuterium Oxide (D, 99.9%)

Catalog #: DLM-4-MG

Formula: D₂O

Molecular Weight: 20.03

CAS # : 7789-20-0

Batch # : PR-20169

Production Completed: 03/06/2009

Test	Reference/Method	Specification	Results
Bromide	IC	< 1 mg/L	< 0.2 mg/L
Calcium	ICP-MS	< 0.1 mg/L	0.023 mg/L
Chloride	IC	< 1 mg/L	< 0.2 mg/L
Conductivity	CIL	< 15 µs/cm	1.0 µs/cm
Fluoride	IC	< 1 mg/L	< 0.2 mg/L
Bioburden	EP 2.6.12B	< 10 CFU/ml Std Plate Ct. < 10 CFU/ml Mold < 10 CFU/ml Yeast	< 1 CFU/ml Std Plate Ct. < 1 CFU/ml Mold < 1 CFU/ml Yeast
Iodide	ISE	< 1 mg/L	< 0.1 mg/L
Heavy Metals (Sb, As, Bi, Cd, Cu, Fe, Pb, Hg, Mo, Ag, Sn)	ICP MS	< 0.1 ppm	0.00023 mg/L (Sb) <0.00006 mg/L (As) 0.000034 mg/L (Bi) 0.00019 mg/L (Cd) <0.0001 mg/L (Cu) <0.0005 mg/L (Fe) <0.00004 mg/L (Pb) <0.00004 mg/L (Hg) <0.0003 mg/L (Mo) <0.00001 mg/L (Ag) 0.000043 mg/L (Sn)
Potassium	ICP-MS	< 0.1 mg/L	0.027 mg/L
Magnesium	ICP-MS	< 0.1 mg/L	0.014 mg/L
Sodium	ICP-OES	< 5 mg/L	0.4 mg/L
Ammonium	IC	< 1 mg/L	< 0.1 mg/L
Nitrate	IC	< 1 mg/L	< 0.2 mg/L
pH	CIL	5.0-8.0	6.4
Phosphorous	ICP-OES	< 1 mg/L	< 0.2 mg/L
Residue on Evaporation	Std. Methods (21 st Edn.) 2540B	< 0.004%	< 0.004%
Sulfur Content	ICP-OES	< 1 mg/L	< 0.2 mg/L
Endotoxin	cUSP <85>	< 0.125 Eµ/mL	< 0.125 Eµ/mL
Carbon	CIL- Total Organic Carbon	< 5 mg/L	0.5 ppm
Identification	¹ H-NMR	Conforms	Conforms
Isotopic Enrichment	¹ H-NMR	> 99.88%	99.93%

Store at room temperature away from light and moisture.

Certified:

Thomas Dorsey

Quality Assurance

03/09/2009

Date

Appendix 8: Patient information leaflet and consent form for the study of breath analysis of deuterium abundance following IV D₂O to estimate lung water volume.

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Patient Information leaflet (version 2.0 10th August 2008)

Title of study: Evaluation of breath deuterium kinetics and the effects of posture following central injection of Deuterium Oxide as an estimate of total lung water (TLW) content in haemodialysis patients.

Short title: Using breath test to measure lung water volume

Invitation to participate

We are inviting you to take part in a clinical study. Please take time to read and consider the following information carefully. You can discuss this with whomever you wish. If you are unclear of any issues please feel free to contact your consultant or your named nurse.

What is the purpose of the study?

One of the purposes of haemodialysis treatment is to remove excess fluid. **It is known that excess fluid in the body is associated with poorer outcome. While having accumulation of fluid in the legs may not cause much problem, too much fluid in the lungs can make patients breathless and cause harm to general health. The challenge for the clinical staffs is to find out how much excess fluid is present in the lungs so that we can plan future treatment better for the patients. Currently we do not have any lung water measurement that is easy to use and acceptable to patients. However, we have developed a simple and acceptable breath test which allows us to measure total body water precisely and we are now trying to apply this to measure lung water volume.** We want to find out how best to apply the breath test in order to obtain consistent and accurate results. We also want to make this as easy as possible for patients without causing too much inconvenience.

Why have I been chosen?

You have been chosen because you are treated with haemodialysis.

Do I have to take part?

No. It does not matter if you are unable to take part for whatever reason. Your usual treatment will not be affected

What will happen to me if I take part and what do I have to do?

You will be asked to sign a consent form. We will arrange a visit for you to come to hospital at your convenience. This can be before, after or in between your dialysis sessions. The visit will last approximately 1 hour. During that time you will have various tests to assess your fluid status. These include the following:

-General measurements such as height and weight

-Deuterium (heavy water) injection. This is a naturally occurring form of water present in tap water which is quite safe and not radioactive. We will use the vascular access that you normally use for your dialysis treatment. In the case of a fistula a small butterfly needle will be used for the purpose of injection.

-Breath test before and after heavy water injection. You will be asked to breathe in and out normally into a flexible tube which connects to a machine. It will analyse the amount of heavy water present in your breath after the injection. You may be asked to perform this procedure several times in lying and sitting positions so that the results can be compared.

-Bioelectrical impedance test. This test is similar to ECG recording where sticky pads are placed on your wrist and ankle and a very small electrical current is passed through the body for approximately 2 to 3 minutes. (You will not feel this) This is a very quick and easy way to calculate the amount of body fluid.

You will be asked to repeat the tests at a later date. This is so that we can compare the results to ensure consistency. A separate consent form will be used for this purpose.

Can I withdraw from the study at a later date?

Yes. You are free to withdraw from the study at any stage even if you have agreed initially.

What are the side effects or risks of taking part?

We do not anticipate any significant side effects or risks of taking part in the study. In addition, we are doing everything we can to make the study as safe as possible such as using very small needle for injection to reduce the chance of bleeding and infection. The study will be carried out by a trained and qualified research doctor.

What are the possible benefits of taking part?

None

What if new information becomes available?

If any new information comes to light to suggest any harm to patients the study will be stopped.

What about travelling costs?

Any travelling costs incurred by the study will be reimbursed.

What happens when the research study stops?

When the study is completed, we will analyse the results and use these to plan the next stage of our research.

What happens to the results of the research study?

It will be presented at scientific meetings and published in international research journals. These results will also help in designing future studies.

Who is organising and funding the research?

This research is organised by Professor Simon Davies, Professor of dialysis medicine of University Hospital North Staffordshire. **The project is jointly funded by Renal Discovery Extramural Grant (Baxter), North Staffordshire Medical institute and the Wellcome trust.**

Will my taking part in this study be kept confidential?

Yes. All data collected from this study will be kept anonymous.

Who has reviewed the study?

This study has been reviewed by experts in dialysis treatment who are independent of the study and has been approved by the local peer review and the local ethics committees.

Contact for further information

If at any stage you require more information please do not hesitate to contact your own named nurse, or from Professor Simon Davies who is the consultant responsible for the study by calling his secretary at 01782554164 or Dr. Julie Wessels (01782554165), the consultant in charge of the haemodialysis treatment programme in your hospital.

Error! Objects cannot be created from editing field codes.

PATIENT CONSENT FORM

Title of study: Evaluation of breath deuterium kinetics and the effects of posture following central injection of Deuterium Oxide as an estimate of total lung water (TLW) content in haemodialysis patients.

Short title: Using breath test to measure lung water volume

I have read the Patient Information Sheet (Version 1.0 dated 9 Feb 2008) and have had the opportunity to discuss any concerns.

I agree to taking part in this study and that my participation is voluntary and that I am free to withdraw from the study at any time without my treatment being affected.

I give permission for information to be taken, in confidence, from my medical records including my date of birth, diagnosis, time on dialysis and the results of any tests done as part of the study.

I understand that I will not benefit financially if this research leads to the development of a new treatment or medical test.

Patient Name (PRINT) _____ Signature _____

Witness Name (PRINT) _____ Signature _____

Date: ___/___/_____

(Version 1.0 dated 9 Feb 2008)

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